R0150A B2

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 June 2001 (28.06.2001)

PCT

(10) International Publication Number
WO 01/45509 A1

(51) International Patent Classification[7]: A01N 43/04, A61K 31/70, C07H 19/00, 19/04, 19/044

(21) International Application Number: PCT/US00/34605

(22) International Filing Date:
19 December 2000 (19.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/172,097	23 December 1999 (23.12.1999)	US
60/173,446	29 December 1999 (29.12.1999)	US
60/175,111	6 January 2000 (06.01.2000)	US
60/190,758	20 March 2000 (20.03.2000)	US
60/226,947	22 August 2000 (22.08.2000)	US
60/226,875	22 August 2000 (22.08.2000)	US
60/233,821	19 September 2000 (19.09.2000)	US
60/233,823	19 September 2000 (19.09.2000)	US
60/233,548	19 September 2000 (19.09.2000)	US
60/233,822	19 September 2000 (19.09.2000)	US
60/235,465	26 September 2000 (26.09.2000)	US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *With international search report.*

— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/45509 A1

(54) Title: COMPOSITIONS AND METHODS FOR L-NUCLEOSIDES, L-NUCLEOTIDES, AND THEIR ANALOGS

(57) Abstract: Nucleoside and nucleotide compounds and their analogs/prodrugs are provided. Particularly contemplated compounds include 1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide, which may be modified and/or phosphorylated. Contemplated compounds may further be combined with other pharmacological compounds, especially including Ribavirin, antibodies, and cytokines. Preferred uses of contemplated compounds include use as an antiviral compound, anti-inflammatory compound, antineoplastic compound, and as a compound to stimulate cellular growth.

COMPOSITIONS AND METHODS FOR L-NUCLEOSIDES, L-NUCLEOTIDES, AND THEIR ANALOGS

This application claims the benefit of U.S. provisional patent application 60/173,446, filed 12/29/1999, U.S. provisional patent application 60/172,097, filed 12/23/1999, U.S. provisional parent application 60/175,111, filed 01/06/2000, U.S. provisional patent application 60/190,758, filed 3/20/2000, U.S. provisional patent application 60/226,947, filed 8/22/2000, U.S. provisional patent application 60/226,875, filed 8/22/2000, U.S. provisional patent application 60/233,821, filed 9/19/2000, U.S. provisional patent application 60/233,823, filed 9/19/2000, U.S. provisional patent application 60/233,548, filed 9/19/2000, U.S. provisional patent application 60/233,822, filed 9/19/2000, and U.S. provisional patent application 60/235,465, filed 9/26/2000, all of which are incorporated herein by reference.

Field of The Invention

The field of the invention is pharmaceutical compositions and uses thereof.

Background of The Invention

There are numerous challenges to a person's health, many of which result from infection or accumulation of toxins in a vital organ, which may further result in an adverse reaction of the immune system towards the infected organ. For example, an infection with the hepatitis C virus (HCV) frequently leads to a persistent inflammatory viral infection in which the organ inflammation may not be immediately attributable to the HCV virus, but rather to an infection induced imbalance in the immune response.

Most known treatments of viral infections may generally be characterized as either direct antiviral treatment or indirect antiviral treatment. In direct antiviral treatment, the virus is targeted with an appropriate direct antiviral drug. For example, patients infected with the HIV virus typically receive a cocktail of drugs to block the virus propagation, and various classes for direct antiviral treatment are known in the art. For example, some direct antiviral drugs block the reverse transcriptase. Reverse transcriptase (RT) inhibitors are typically nucleoside analogs such as AZT, 3TC, or ddI. Alternatively, non-nucleoside RT inhibitors, including quercetin may be used. *In vitro*, RT inhibitors are typically potent antiviral drugs. However, *in vivo*, and especially during a period of relatively high rate of viral replication, the generation of RT inhibitor resistant virus mutants are problematic.

Other direct antiviral drugs block or interfere with the virus protein processing, and are commonly known as protease inhibitors. Protease inhibitors are typically highly specific towards the viruses' proteolytic enzymes. However, due to their mostly hydrophobic nature, administration at desirable concentrations tends to be problematic. Moreover, development of cross-resistance and severe side effects frequently compound the difficulties arising from the use protease inhibitors. In order to reduce the development of multi-drug resistant virus strains, mixtures of RT inhibitors and protease inhibitors may be prescribed. Although such mixtures are presently employed relatively successfully, the relatively high occurrence of adverse side effects and the potential of generating multi-drug resistant virus strains persist.

In indirect antiviral treatment, the immune response to a viral challenge may be modulated. For example, immunosuppressive drugs may be employed to reduce the inflammatory condition associated with the viral infection, and various immunosuppressing drugs are known in the art. Among other immunosuppressive drugs, cyclosporin A is known as a potent immunosuppressor and is frequently used to repress tissue rejection after organ transplantation. However, the use of cyclosporin A tends to be problematic due to its general immunosuppressing effect, making the patient more prone to new infectious diseases. Furthermore, long-term administration of cyclosporin A is frequently associated with severe side effects, including hirsutism and gingival hyperplasia. Moreover, the bioavailability of cyclosporin A is at least in part dependent on bile, which may pose additional problems in a hepatitis infection.

To overcome at least some of the problems associated with cyclosporin A, Tacrolimus (FK506) may be employed as an immunosuppressing drug. For example, Tacrolimus has found recognition in the treatment of facial atopic dermatitis. Topical administration of the immuno suppressant resulted in significant improvement in 95% of all treated patients [Alaiti, S. *et al.* Tacrolimus (FK506) ointment for atopic dermatitis: A phase I study in adults and children. *J Am Acad Dermatol* 1998; 38(1): 69-76]. Furthermore, Tacrolimus appeared not to permeate through the skin barrier, thereby eliminating problems associated with systemic administration. Although generally well tolerated, treatment with Tacrolimus without generally compromising immunity is limited to topical administration. When systemically administered over prolonged periods, Tacrolimus frequently leads to lymphoproliferative disorders and cardiomyopathy.

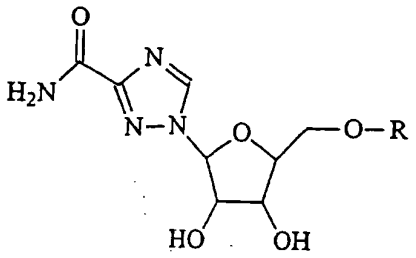
Many known immunosuppressive drugs provide some relief for inflammatory conditions. However, the effects are not organ specific when systemically administered. Consequently, immunity towards exogenous and endogenous challenges such as bacterial and viral infections, neoplastic or malignant cells, etc. is systemically reduced. Thus, the window of a usable concentration of immunosuppressive drugs is defined by the maximum concentration that will not entirely compromise a patient's immune system, and the minimum concentration that will provide at least some desirable effect.

Although various compounds and methods for treatment of infectious and inflammatory diseases are known in the art, all or almost all of them suffer from one or more disadvantages. Therefore, there is a need to provide improved methods and compositions for treatment of those conditions.

Summary of the Invention

The present invention is directed to methods and compositions in which a nucleoside and/or nucleotide drug or its analog is administered to a subject in a concentration or dosage effective to achieve a desired pharmacological or physiological effect.

In one aspect of the inventive subject matter, contemplated compounds have a structure according to formula I, wherein R is H, a PO_3^{2-}, $(PO_3)_2^{3-}$, or $(PO_3)_3^{4-}$group.



Formula 1

Contemplated compounds are optionally further modified with a modifying group that is covalently coupled to the carbonyl atom, and it is further contemplated that compounds according to the inventive subject matter are in a D- or L-configuration.

In another aspect of the inventive subject matter, contemplated compounds are employed to treat a viral infection, and may further be co-administered with a cytokine, preferably IFN-alpha-2b, an antibody, or Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide).

In a further aspect of the inventive subject matter, the selectivity of contemplated compounds with respect to a pharmacological effect in a target cell is increased by modifying the compounds with a modifying group, wherein the modifying group is covalently attached to the drug via a nitrogen atom, and wherein the modifying group is enzymatically removed from the drug in the target cell. Particularly contemplated modifying groups include =NH, and $-N(R_1)(R_2)$ or $=NR_1$, wherein R_1 and R_2 are independently hydrogen, a linear alkyl, a branched alkyl, an alkenyl, an alkynyl, an aralkyl, an aralkenyl, an aralkynyl, or an aryl, and wherein R_1 or R_2 may independently further comprise a nitrogen atom, an oxygen atom, a sulfur atom, or a halogen atom.

In a still further aspect of the inventive subject matter, a method of treating a disease characterized by inflammation of an organ in a patient has a step in which contemplated compounds are administered to a patient at a dosage that causes systemic immunomodulation and not systemic immunosuppression of Type I and Type II responses. This causes immunosuppression of Type I and Type II responses in the organ of the patient due to selective accumulation of contemplated compounds in the organ.

In yet a further aspect of the inventive subject matter, a method of stimulating neuronal growth has a step in which it is recognized that contemplated compounds are effective to stimulate growth of neurons within a given concentration range. In a further step, the compounds are provided to the neurons within the given concentration range.

Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of various embodiments of the invention.

Brief Description of The Drawing

Figures 1A-1C are exemplary compounds according to the inventive subject matter.

Figure 2 is an exemplary synthetic scheme for the synthesis of 1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide.

Figure 3 is an alternative exemplary synthetic scheme for the synthesis of 1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide.

Figure 4 is another exemplary synthetic scheme for the synthesis of 1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide.

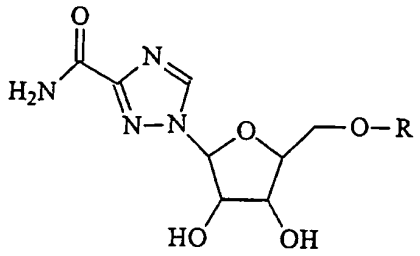
Figure 5 is a flow diagram depicting an exemplary method of organ-targeted immuno-suppression according to the inventive subject matter.

Figure 6 is a flow diagram depicting an exemplary method of stimulating cell growth according to the inventive subject matter.

Detailed Description

Contemplated Compounds

It is generally contemplated that all nucleotides, nucleosides, and their corresponding analogs are suitable for use in conjunction with the teachings presented herein, wherein all of the contemplated compounds may be in their respective L-configuration or D-configuration. However, particularly preferred compounds include phosphorylated and unphosphorylated Levovirin™ (1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide, Structure 1), in which R may be hydrogen, or a phosphorous or sulfur-containing group. Where R is a phosphorous containing group, it is especially preferred that R is a monophosphate, a diphosphate, or a triphosphate as depicted in **Figures 1A-1C**.



Structure 1

Depending on the chemical environment (and especially depending on the pH), it should be appreciated that the phosphate groups may be in their corresponding mono-, di-, tri-, and tetra-protonated forms, and it should also be appreciated that when the phosphate groups are partially or completely deprotonated, salts may be formed with one or more mono- or multivalent cations. Especially contemplated cations are alkaline metal ions and alkaline earth metal ions such as Mg^{2+}, Cs^{2+}, Na^{+}, etc.

In alternative aspects of the inventive subject matter, R may also be a PO_3^{2-}, $(PO_3)_2^{3-}$, or $(PO_3)_3^{4-}$ group in which one or more than one oxygen is replaced with a sulfur atom. While phosphate groups are generally preferred substitutents for R, other chemical groups may also be

employed, and particularly contemplated groups include mono-, or polyanionic groups, preferably with a tetragonal geometry. Thus, contemplated compounds especially include modified and unmodified phosphorylated Levovirin™. Furthermore, it should be appreciated that contemplated compounds may also have a sugar moiety in the D-configuration, and an especially contemplated compound with a sugar in D-configuration is Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide).

It should further be appreciated that at least some of the contemplated compounds exhibit a direct antiviral effect (*i.e.* contemplated compounds immediately inhibit viral propagation). Since most organisms possess phosphatases in various compartments, it is contemplated that the compounds according to the inventive subject matter may be gradually dephosphorylated, and one or more than one phosphate group may be removed at a time. For example, a triphosphorylated compound may be converted into a diphosphorylated, or monophosphorylated compound, or a diphosphorylated compound may be converted into Levovirin™ in a single reaction.

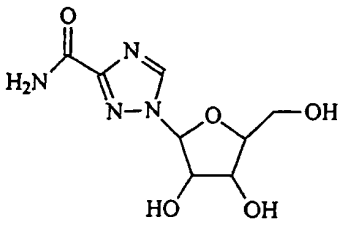
With respect to dephosphorylation of phosphorylated Levovirin™, it is particularly contemplated that the mode of antiviral action shifts from a direct antiviral effect to an indirect antiviral effect. The shift from a direct antiviral response to an indirect antiviral response is particularly advantageous, because even though the contemplated compounds are metabolized they retain anti-viral action over an extended period. Therefore, it should be appreciated that the mode of action of contemplated compounds, and particularly phosphorylated Levovirin™, is actually at least bimodal – comprising a direct antiviral effect portion and an indirect antiviral effect portion.

With respect to the rate of dephosphorylation it is contemplated that phosphorylated Levovirin™ is dephosphorylated at a considerably slower rate than phosphorylated Ribavirin, an effect that is contemplated to be due to the L-configuration of the ribose in Levovirin™. With respect to the compartment or organ where dephosphorylation may occur, it is contemplated that dephosphorylation preferably takes place in the liver, however, other organs and compartments, including kidney, neuronal cells, and blood stream are also contemplated.

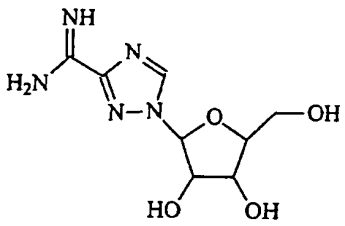
It should be especially appreciated that all known prodrug forms of contemplated compounds are appropriate for use in conjunction with the teachings presented herein, and particularly contemplated prodrug forms include covalent modifications that may be

enzymatically (*e.g.*, by a aminohydrolase, an oxidoreductase, or a transferase) removed from contemplated compounds. Exemplary suitable prodrug forms are described in U.S. Patent Application number 09/594,410, filed 06/16/00, incorporated herein by reference, and in "Prodrugs" by Kenneth B. Sloan (Marcel Dekker; ISBN: 0824786297), or "Design of Prodrugs" by Hans Bundgaard (ASIN: 044480675X), also incorporated herein by reference.

Further, especially contemplated examples of suitable prodrugs include prodrugs formed by addition of a nitrogen-containing group to the carboxamide moiety of Levovirin™, which may be especially advantageous where contemplated compounds are preferentially directed to the liver. For example, the inventors have discovered (unpublished results) that the specificity of Levovirin™ with respect to its pharmacological effect in hepatocytes can be improved by modifying Levovirin™ with a nitrogen-containing modifying group that is selectively removed in hepatocytes. Structure 2 below shows Levovirin™, and Structure 3 shows Levovirin™ modified at the carboxamide group to form a carboxamidine group.



Structure 2



Structure 3

It is particularly contemplated that a modification of Levovirin™ with a (preferably nitrogen-containing) modifying group that can be selectively removed in a target cell (*e.g.*, a hepatocyte) will (1) increase the selectivity of Levovirin™ with respect to the target cell, thereby (2) reducing the overall dosage to achieve a desired effective concentration, and (3) reduce potential toxicity in non-target cells. It is further contemplated that the modifying group is covalently bound to the carbonyl atom of the carboxamide group.

In further alternative aspects, the nitrogen containing modifying group need not be limited to an =NH group, but may also include various primary and secondary amines. It is generally contemplated that suitable modifying groups have the structure $-N(R_1)(R_2)$ or $=NR_1$, wherein R_1 and R_2 are independently hydrogen, linear or branched alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, or aralkynyl, aryl, all of which may further comprise heteroatoms including nitrogen, oxygen, sulfur, or a halogen. It is especially preferred, however, that alternative

modifying groups are enzymatically removable from Levovirin™, and particularly contemplated enzymes include aminohydrolases such as liver deaminases (*e.g.*, adenosine or cytosine deaminase), liver deamidases (*e.g.*, aryl deamidase) and liver transaminases (glutamate-pyruvate transaminase).

Although not limiting to the inventive concept presented herein, it is contemplated that the modifying group may inactivate Levovirin™, or prevent subsequent activation once the modified Levovirin™ is presented to a non-target cell. On the other hand, the nitrogen-containing modifying group may also prevent metabolic activation of the modified Levovirin™. With respect to the step of modifying Levovirin™, it is contemplated that the modification may comprise an organo-synthetic modification, an enzymatic modification, or a *de-novo* synthesis to produce the modified Levovirin™.

With respect to the enzymatic removal of the modification group, it is contemplated that, depending on the type of the target cell and the modifying group, the enzymatic removal may vary considerably. Enzymatic removal may include enzymes from various classes, including hydrolases, transferases, lyases, and oxidoreductases, and particularly preferred subclasses are adenosine and cytosine deaminases, arginases, transaminases, and arylamidases. It should further be appreciated that contemplated enzymes for the enzymatic removal of the modification group may exclusively be expressed in the target cells, however, in alternative aspects of the inventive subject matter appropriate enzymes may also be expressed in cells other than the target cells, so long as the enzyme is not ubiquitously expressed in all cells in a cell containing system. It should further be appreciated that contemplated enzymes are preferably natively expressed (*i.e.*, are non-recombinant) in the respective target cells under normal and/or pathological conditions. For example, it is known that glutamine-pyruvate transaminase is constitutively expressed with relatively high selectivity in liver cells, and may therefore be a suitable enzyme for removal of a modification group. Alternatively, it is known that cytosine deaminase is expressed in relatively high quantities in colon cancer cells, but not, or only in minor quantities in normal colon cells.

Synthesis of contemplated compounds

It is generally contemplated that all known methods of synthesis for D-nucleotides, D-nucleosides, and their respective analogs may be adapted for the synthesis of contemplated compounds in the L-configuration (*e.g.*, by replacing the sugar moiety in D-configuration with a

sugar moiety in the corresponding L-configuration). An exemplary synthetic scheme for the synthesis of Levovirin™ (1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide) is depicted in **Figure 2**.

Synthesis of 1,2,3,5-Tetra-O-acetyl-β-L-ribofuranose (1)

To a stirred solution of L-ribose (50.0g, 333.33mmol) in anhydrous methanol (500ml) at room temperature was added freshly prepared dry methanolic HCl (40ml, prepared by bubbling dry HCl gas into methanol at 0°C to a weight increase of 4g) via syringe during a 15 min. period under argon atmosphere. After the addition of methanolic HCL, the reaction mixture was allowed to stir at room temperature for 3-4h. Dry pyridine (100ml) was added and evaporated to dryness under high vacuum below 40°C. This process was repeated a second time with additional dry pyridine (100ml). The residue was dissolved in dry pyridine (250ml) and cooled in an ice bath to 0°C under argon atmosphere. To this cold stirred solution was added acetic anhydride (100ml) via a dropping funnel during a 15 min. period. After the addition of acetic anhydride, the reaction was allowed to stir at room temperature under exclusion of moisture for 24h. The reaction mixture was evaporated to dryness. The residue was partitioned between ethyl acetate (400ml) and water (400ml), and extracted in EtOAc. The aqueous layer was extracted again with EtOAc (100ml). The combined EtOAc extract was washed with water (400ml), saturated $NaHCO_3$ (2x 300ml), water (300ml), and brine (200ml). The organic extract was dried over anhydrous Na_2SO_4, filtered, and the filtrate evaporated to dryness. The residue was co-evaporated with dry toluene (2x150ml) at high vacuum. The dried oily residue (92g, 95%) was used as such for the following reaction without further characterization.

The syrup (92g) from the above reaction was dissolved in glacial acetic acid (300ml) and treated with acetic anhydride (75ml) at room temperature. The solution was cooled to 0-5°C in an ice bath under argon atmosphere. Concentrated H_2SO_4 (21ml) was added slowly during a 15min. period. After the addition of H_2SO_4, the reaction mixture was stirred at room temperature for 14h, poured on crushed ice (500g), and stirred until the ice melted. Water (500ml) was added and extracted with $CHCl_3$ (2x300ml). The chloroform extract was washed with water (3x400ml), saturated $NaHCO_3$, (2x300ml), water (200ml) and brine (200ml). The washed organic extract was dried over anhydrous $MgSO_4$, filtered and evaporated to dryness to give an oily residue (99g). The residue was co-evaporated with dry toluene (200ml) and dissolved in ethyl ether

(200ml), which upon cooling at 10°C for a day produced colorless crystals. The crystalline solid was filtered, washed with hexanes, ether (2:1, 50ml), and dried to give a 60.5g product.

Synthesis of Methyl-1-(2,3,5-tri-O-acetyl-β-L-ribofuranosyl)-1,2,4-triazole-3-carboxylate (3) and Methyl-1-(2,3,5-tri-O-acetyl-β-L-ribofuranosyl)-1,2,4-triazole-5-carboxylate (4)

A mixture of methyl-1,2,4-triazole-3-carboxylate (25.4g, 200mmol), 1,2,3,5-tetra-O-acetyl-β-L-ribofuranose (63,66g, 200mmol) and bis(p-nitrophenyl)phosphate (1g) were placed in an RB flask (500ml). The flask was placed in a preheated oil bath at 165-175°C under water aspirator vacuum with stirring for 25min. The acetic acid displaced was collected in an ice-cold trap that was placed between the aspirator and the RB flask. The flask was removed from the oil bath and allowed to cool. When the temperature of the flask reached roughly 60-70°C, EtOAc (300ml) and saturated $NaHCO_3$ (150ml) were introduced, and extracted in EtOAc. The aqueous layer was extracted again with EtOAc (200ml). The combined EtOAC extract was washed with saturated $NaHCO_3$ (300ml), water (300ml) and brine (200ml). The organic extract was dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The residue was dissolved in EtOH (100ml) and diluted with MeOH (60ml), which on cooling at 0°C for 12h produced colorless crystals. The solid was filtered, washed with minimum cold EtOH (20ml), and dried at high vacuum over solid NaOH to give 60g (78%). The filtrate was evaporated to dryness and purified on a silica column using $ChCl_3$-> EtOAc (9:1) as the eluent. Two products were isolated from the filtrate: fast moving product 8.5g (11%) and slow moving product 5g(6.5%). The slow moving product matched with the crystallized product. The fast moving product was found to be (4) and obtained as foam. The combined yield of (3) was 65g (84%).

Synthesis of 1-β-Ribofuranosyl-1,2,4-triazole-3-carboxamide (5)

Methyl-1-(2,3,5-tri-O-acetyl-β-L-ribofuranosyl)-1,2,4-triazole-3-carboxylate (62g, 161mmol) was placed in a steel bomb and treated with freshly prepared methanolic ammonia (350ml, prepared by passing dry HCL gas into dry methanol at 0°C until saturation) at 0°C. The steel bomb was closed and stirred at room temperature for 18h. The steel bomb was then cooled to 0°C, opened, and the content evaporated to dryness. The residue was treated with dry ethanol (100ml) and evaporated to dryness. The residue obtained was triturated with acetone to give a solid, which was filtered and washed with acetone. The solid was dried overnight at room

temperature and dissolved in a hot EtOH (600ml) and water (10ml) mixture. The volume of the EtOH solution was reduced to 150ml by heating and stirring on a hot plate. The hot EtOH solution on cooling provided colorless crystals, which were filtered, washed with acetone, and dried under vacuum. Further concentration of the filtrate gave additional material. The total yield was 35g (89%).

In an alternative aspect of the inventive subject matter, it is contemplated that the synthesis of Levovirin™ may also employ one or more enzymatic conversions. For example, the acetylation of L-ribose may be performed with a suitable acetyl-transferase (*e.g.*, EC 2.3.1.xx). In another example, the formation of the carboxamide group from the corresponding methylester may be facilitated by a single or dual-enzyme system involving an esterase (*e.g.*, EC 3.1.1.xx) and/or aminotransferase (*e.g.*, EC 2.6.1.xx). In still another example, Levovirin™ may be enzymatically converted into the corresponding mono-, di-, or triphosphate (e.g., EC 3.1.3.xx or EC 3.1.4.xx).

It is still further contemplated that various catalysts other than bis(p-itrophenyl)phosphate in quantities other than 1g may be utilized. Changing the amount (*i.e.*, the molar fraction) of the catalyst may advantageously increase the selectivity of the reaction towards a higher yield of the desired N_1 isomer (L-ribose coupled to the N_1 atom of the triazole ring) over the N_2 isomer. For example, appropriate amounts of bis(p-nitrophenyl)phosphate include amounts between 3-30mmol, and more. Alternatively, where appropriate, amounts lower than 3mmol (0.3mmol – 2.99mmol) may be included. In further alternative aspects of the inventive subject matter, the catalyst need not be limited to bis(p-nitrophenyl)phosphate, and alternative catalysts include p-toluenesulfonic acid, trichloro acetic acid, and p-nitrobenzoic acid.

With respect to the reaction temperature, it is particularly contemplated that lower temperatures may further increase the selectivity of the reaction towards a higher yield of the desired N_1 isomer over the N_2 isomer. Therefore, it is contemplated that appropriate temperatures for the coupling reaction between the triazole moiety and the ribose moiety include temperatures between about 155-165°C, more preferably between 145-165°C, and most preferably between 130-165°C.

In further alternative aspects of the inventive subject matter, it is contemplated that the selectivity of the reaction towards a higher yield of the desired N_1 isomer over the N_2 isomer may also be favorably influenced by a chemical modification of the methyl-1,2,4-triazole-3-

carboxylate. Chemical modifications include formation of a complexing structure that involves the N_2 atom, steric hindrance, and direct chemical modifications of the N_2-atom. For example, the free electron pair in the N_2 atom of the triazole moiety and an electron donor in a modified carboxylate group may be employed to complex a metal ion, thereby reducing the availability of the N_2 atom for coupling with the ribose moiety. In another example, the carboxylate group in the methyl-1,2,4-triazole-3-carboxylate may be modified with a relatively bulky group that preferentially and sterically blocks or reduces reactions occurring at the N_2 atom. Alternatively, the N_2 atom may be directly modified by a protecting group, and suitable protecting groups include t-Boc, and benzyl.

Still further, it is contemplated that a higher yield of the desired N_1 isomer over the N_2 isomer may also be achieved using enzymatic synthesis in which the ribose moiety (or an L-ribonucleotide) and a modified or non-modified methyl-1,2,4-triazole-3-carboxylate serve as a substrate for a ribosyltransferase (*e.g.*, EC 2.4.2.5 or EC 2.4.2.6).

Alternatively, Levovirin™ may be synthesized via coupling of a protected L-ribose to a 1,2,4-triazole-3-nitrile, with subsequent conversion of the nitrile group to the carboxamide as shown in **Figure 3**. In a still further alternative synthesis, the coupling of the triazole moiety with the ribose moiety may also be achieved in a reaction in which a (*e.g.*, benzyl protected) ribose has an $-NHNH_2$ group coupled to the C_1 atom, that is reacted with the N_1 atom of the triazole carboxylate, wherein the triazole carboxylate is subsequently converted to the carboxamidine as depicted in **Figure 4**.

With respect to the synthesis of prodrug forms of contemplated L-nucleotides, L-nucleosides and their respective analogs, it should be appreciated that a particular synthetic scheme will generally depend on the structure of the particular compound. However, all manners of synthesis are considered suitable and contemplated synthetic schemes include *in vitro* synthesis, enzymatic synthesis, *in-vivo* conversions, and any chemically reasonable combination thereof. Exemplary synthetic schemes for the formation of contemplated prodrugs are described in U.S. Patent Application number 09/594,410 (*supra*).

Where contemplated L-nucleotides, L-nucleosides and their respective analogs are phosphorylated, it is contemplated that all manners of incorporating a phosphate group into a nucleotide, nucleoside or their respective analogs are suitable. Conversion of contemplated nucleosides to their corresponding phosphorylated forms can be achieved synthetically (Hughes,

B.G. et al, (1983); 2',5'-oligoadenylated and related 2',5-oligonucleotide analogues. 1. Substrate specificity of the interferon-induced murine 2',5'-oligoadenylate synthetase and enzymatic synthesis of oligomers. Biochemistry, 22: 2116-2126). However, various alternative methods are also contemplated and include enzymatic phosphorylation (see *e.g.*, Van Rompay, A.R., et al (2000); Phosphorylation of nucleosides and nucleoside analogs by mammalian nucleoside monophosphate kinases; Pharmacol. Ther. 87(2-3):189-198), and organochemical phosphorylation in aqueous media (Schwartz, A. and Ponnamperuma, C. (1968); Phosphorylation of adenosine with linear polyphosphate salts in aqueous solution (Nature 218, 443).

Uses of Contemplated Compounds

It should generally be recognized that contemplated compounds may be employed in any treatment or therapy of a system that positively responds to administration of contemplated compounds. However, it is particularly preferred that contemplated compounds may be employed in antiviral treatments (as a direct antiviral compound and/or as an indirect antiviral compound), in treatments to modulate the immune system, and in treatments to stimulate cellular growth. Further, particularly contemplated uses include administration of contemplated compounds in antineoplastic treatments.

Antiviral treatments

It is generally contemplated that compounds according to the inventive subject matter may be employed as a direct and/or indirect antiviral agent in a viral infection. It is particularly contemplated that a method of treating a viral infection in a patient comprises a step in which composition is administered to the patient at a dosage effective to inhibit viral propagation (*i.e.*, a process involving a host cell in which one or more than one virus causes the host cell to produce one or more copies of the virus, wherein the term "to produce" refers to nucleotide synthesis, protein processing, and protein assembly), wherein the composition comprises at least one the contemplated compounds, and preferably at least one of a compound according to Structures 1 and 3. Preferred dosages are in the range of between 5-2500mg/day, and more preferably between 50-500mg/day. However, alternative dosages, routes, schedules and

formulations are also contemplated, and suitable alternative administrations are described below. While the use of contemplated compounds is not restricted to a particular virus in a particular viral infection, especially contemplated viral infections are an HIV infection, an HCV infection, an HBV infection, a RSV infection, an influenza virus infection, and a parainfluenza virus infection.

Peripheral blood mononuclear cells (PBMCs) are currently used to study several different infections, such as Hepatitis C, HIV, Hepatitis B and varieties of the Herpes virus. (*Antivir. Chem. Chemother.* 2000 July; 11(4): 291-301; *J. Infect. Dis.* 1998 Oct; 178(4): 1189-92; *Virology* 2000 Mar.; 268(**1**):12-60.) PMBCs are infected with the desired virus, and the cell lines are then studied for relevant information on how particular drugs interact with the PBMCs and how the infected PBMCs act over time or under different environmental conditions. Based on the studies conducted with infected PBMCs, models can be generated that show the effects of particular pharmaceuticals, environments, and/or conditions on the PBMC-Virus infected cells.

The present inventors have discovered (unpublished results) that Ribavirin shows a positive response against PBMC-HIV infected cells. Surprisingly, Levovirin™ also shows a similar immunomodulatory profile against PBMC-HIV infected cells despite the body's lack of enzymes necessary to phosphorylate the Levovirin™ present in the patient. Based on the above observations, along with other related information and tests, it is contemplated that Levovirin™, phosphorylated Levovirin™, and modified Levovirin™ according to Structure 3 can be utilized in the treatment of HIV and related viruses.

Immunomodulation

In **Figure 5**, a method of organ-targeted immunosuppression 500 has a first step 510 in which a drug is provided that reduces both a Type 1 response and a Type 2 response when administered above an immunosuppressive concentration, and increases the Type 1 response relative to the Type 2 response when administered below the immunosuppressive concentration, wherein the drug accumulates preferentially in a target organ. In a subsequent step 520, the drug is administered to a patient in a dosage effective to accumulate the drug in the target organ to the immunosuppressive concentration. Consequently, it is contemplated that a method of treating a disease characterized by liver inflammation in a patient may comprise one step in which a

compound is provided, wherein the compound comprises Levovirin, phosphorylated Levovirin™, a modified phosphorylated Levovirin™, or a modified Ribavirin (*supra*). In a further step, the compound is administered to the patient at a dosage that (a) causes systemic immunomodulation and not systemic immunosuppression of Type I and Type II responses, and (b) causes immunosuppression of Type I and Type II responses in the patient's liver.

The term "immunosuppression" refers to an event in which T and/or B cell clones of lymphocytes are depleted in size or suppressed in their reactivity, expansion or differentiation. Immunosuppression may thereby arise from activation of specific or nonspecific T suppressor lymphocytes of either T or B clones, or by drugs that have generalized effects on most or all T or B lymphocytes. For example, Cyclosporin A and FK506 act relatively specifically on T cells, while alkylating agents such as cyclophosphamide are less specific in their action.

As used herein, the term "cytokine" refers to a group of soluble proteins and peptides which act as humoral regulators at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. Cytokines also mediate interactions between cells directly and regulate processes taking place in the extra-cellular environment.

As further used herein, the term "lymphokines" refers to a subset of cytokines produced by helper T cells, and are generally considered to fall into two subclasses, Type 1 and Type 2. Type 1 cells produce interleukin 2 (IL-2), tumor necrosis factor (TNFα) and interferon gamma (IFNγ), and are responsible primarily for cell-mediated immunity such as delayed type hypersensitivity and antiviral immunity. In contrast, Type 2 cells produce interleukins, IL4, IL-5, IL-6, IL-9, IL-10, and IL-13, and are primarily involved in assisting humoral immune responses such as those seen in response to allergens (*e.g.* IgE and IgG4 antibody isotype switching).

Therefore, the terms Type 1 and Type 2 "responses" are meant to include the entire range of effects resulting from induction of Type 1 and Type 2 lymphocytes, respectively. Among other things, such responses include increased production of the corresponding cytokines, increased proliferation of the corresponding lymphocytes, and other effects associated with increased production of cytokines, including motility effects. A Type 1 response is generally characterized by an increase in IL-2, TNF-α, and IFN-γ, whereas a Type 2 response is typically characterized by an increase in IL4, IL-5, IL-6, and IL-10.

As still further used herein, the term the drug "accumulates preferentially" in a target organ refers to a selective mechanism of a target organ resulting in an increased net uptake or retention of the drug into the target organ relative to other tissue or organs. The mechanism may thereby include active import via transporters, receptors, vesicles, etc, but may also be based on physicochemical principles, including pH dependent charge of the drug, different solubility of the drug in environments with altered ionic strength, chemical or enzymatic modification within a target organ or target cell, and so forth.

In a preferred aspect, the drug is Ribavirin, which is provided to a patient with an HCV (Hepatitis C Virus) infection, and Ribavirin is orally administered to the patient in a single dosage of 600mg/day for a period of 180 days. A single dosage of 600mg/day is generally below a systemic immunosuppressive concentration, however is effective to preferentially accumulate in the liver. Thus, the concentration of Ribavirin in the target organ (here: the liver) will significantly increase and reach an immunosuppressive concentration in the liver. Ribavirin is known to increase a Type 1 response relative to a Type 2 response, and to reduce the Type 1 and Type 2 response at relatively high concentrations. Examples are set forth in International Patent Application Number PCT/US98/00634 filed on January 13, 1998, incorporated herein by reference.

In alternative aspects of the inventive subject matter, the drug need not necessarily be limited to Ribavirin, and alternative drugs include contemplated compounds (*supra*), particularly modified and unmodified Levovirin™ and phosphorylated Levovirin™. Further alternative drugs include contemplated compounds so long as alternative compounds reduce both a Type 1 response and a Type 2 response at an immunosuppressive concentration, and increase the Type 1 response relative to the Type 2 response below the immunosuppressive concentration.

With respect to the patient, various viral infections other than HCV infection are also contemplated, including infections with arboviruses. Consequently, the target organ is not restricted to the liver, but may also include other organs such as the brain, the lung, the spleen, the thymus, the kidneys, etc. In general, it is contemplated that the disease that can be treated with the method according to the subject matter presented herein, will depend on the drug's specific accumulation pattern (*i.e.* in which organ the drug preferentially accumulates). For example, Ribavirin and Levovirin™ both preferentially accumulate in the liver, and reduce both the Type 1 and Type 2 response above an immunosuppressive concentration. Therefore, diseases

in which suppression of an immune response in the liver is desirable are especially contemplated, and include hepatitis C, autoimmune/lupoid hepatitis, liver transplant recipients, etc.

It should be especially appreciated that the method according to the inventive subject matter is not designed to provide a direct antiviral treatment, but is designed to at least partially suppress an immune response in an organ that is infected with a virus. Organ targeted immune suppression is contemplated to be especially advantageous in hepatitis C, where the organ damage is not immediately attributable to the HCV virus, but rather to an infection-induced imbalance between a Type 1 response and a Type 2 response. Therefore, a method of treatment with a drug that reduces specifically both the Type 1 and Type 2 response in the liver of a patient infected with HCV is contemplated to prevent hepatic damage prophylactically as well as in therapeutic approach. Since Ribavirin and Levovirin™ both have excellent tolerability in humans, long-term prophylaxis, and long-term treatment are particularly advantageous.

With respect to the administration (route, dosage, schedule, term, etc.) of Ribavirin or alternative contemplated compounds, the same considerations as described below apply. It is further contemplated that Ribavirin or alternative contemplated compounds can be employed in a general health setting, as opposed to a clinical, therapeutic setting. Consequently, it is contemplated that Ribavirin or alternative contemplated compounds may also be used to improve digestion. For example, one or more of the compounds may be taken by an individual suffering from poor digestion – whether the poor digestion is due to liver conditions such as Hepatitis B or C infections, or indeed any other conditions characterized by liver inflammation. In such instances, digestion can be improved by having the person take Ribavirin or a Ribavirin like compound below an amount normally producing systemic immunosuppression, but in an amount that accumulates in the liver to a concentration that produces immunosuppression in the person's liver.

Another example of a non-clinical, non-therapeutic use is for a person to take Ribavirin or alternative contemplated compounds as a means of improving skin color. It is particularly contemplated that skin color can be improved by having the person take Ribavirin or alternative contemplated compounds below an amount normally producing systemic immunosuppression, but in an amount that accumulates in the liver to a concentration that produces immunosuppression in the person's liver.

In all of these methods it is particularly contemplated to utilize Ribavirin (1-(5-Deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide) or Levovirin™ (1-(5-Deoxy-β-L-ribofuranosyl)-1,2,4-triazole-3-carboxamide), or any of their mono-, di-, or tri-phosphorylated forms. The amount taken or administered is preferably sufficient to produce a systemic immunomodulation of Type I and Type II responses, and a suppression in the liver of both Type I and Type II responses. Especially preferred amounts are between about 300mg/day and about 800mg/day, although in some individuals the range may be as low as about 50-100 mg/day up to as high as 2000-2400 mg/day. Effects on other organs are also contemplated, including the brain or other organs in which Ribavirin is known to significantly accumulate.

Stimulation of neuronal growth

As used herein, the term "stimulating neuronal growth" refers to any process in which cell growth and/or division is either initiated from a resting cell, or accelerated in a growing and/or dividing cell, wherein "neuronal" refers to all cells that are directly or indirectly involved in the propagation of cognitive, sensory or motor signals. For example, neurons are contemplated to be directly involved in signal propagation, while myelin sheath cells or glia cells are indirectly involved by virtue of their insulating function or structural/metabolic support to a neuron. Similarly, receptors are also considered neuronal cells under the scope of this definition. In contrast, cells forming the inner and outer layer of the dura are not considered neuronal cells, since they are not directly or indirectly involved the propagation of cognitive, sensory or motor signals.

The inventors surprisingly discovered that Ribavirin is effective to stimulate neuronal growth, and the inventors further contemplate that the various phosphorylated analogs of Ribavirin may also be effective to stimulate such growth. It is still further contemplated that Levovirin™ and its phosphorylated analogs may be effective in a similar manner. In a particular experiment, it has been recognized that Levovirin™ is effective to stimulate growth of unipolar neuronal cells *in vitro* within a concentration range of 0.5μM to 500μM. Consequently, addition of Levovirin™ to a culture medium at a concentration of about 5.0μM can be employed to stimulate growth of unipolar neuronal cells.

It should further be appreciated that methods of stimulating neuronal growth need not be limited to unipolar neuronal cells, but may include various alternative cells, including bipolar and multipolar neuronal cells. Furthermore, it is contemplated that in alternative aspects of methods of stimulating neuronal growth, individual cell types may be targeted in a population of diverse neuronal cells. For example, unipolar, bipolar and multipolar neuronal cells may be targeted in complex neuronal structures such as the brain, the spinal chord, or the eye. Therefore, neurons may be part of a neuronal tissue that includes at least four of the following cell types: an astrocyte, a dendrocyte, a myelin sheath cell, a glia cell, a unipolar neuronal cell, a bipolar neuronal cell, a multipolar neuronal cell, and a receptor cell.

Consequently, contemplated methods are not necessarily limited to stimulating neuronal growth in cell culture. In further alternative aspects of the inventive subject matter, it is contemplated that cells may be stimulated in a tissue culture, and it is particularly contemplated that neuronal cells may be stimulated *in vivo*. *In vivo* stimulation of neuronal growth may advantageously be utilized as a prophylactic treatment, or a therapeutic treatment. For example, contemplated methods according to the inventive subject matter may be utilized for prevention of demyelinating disorders or neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease, or as a preventative treatment prior to operative procedures in a patient. Contemplated therapeutic treatments include reversion or attenuation of asphyxial, traumatic, toxic, infectious, degenerative, metabolic, ischemic or hypoxic insults.

Consequently, as depicted in **Figure 6**, a method 600 of improving coordination in a patient has a first step 610 in which it is recognized that phosphorylated or unphosphorylated Ribavirin or Levovirin™ is effective to stimulate growth of neurons *in vivo* within a given concentration range. In a subsequent step 620, the patient takes an amount of phosphorylated or unphosphorylated Ribavirin or Levovirin™ that is effective to stimulate growth of at least some of the person's neurons. Improvement of eye-hand coordination is especially contemplated.

In a preferred method of improving coordination in a person, it is recognized that Ribavirin is effective to stimulate growth of neuronal cells *in vivo* within a concentration range of 0.5μM to 500μM, and Ribavirin is orally administered to a patient suffering from a traumatic injury to the *nervus ischiadicus* in a dosage of 1200mg/day.

With respect to the patient, various conditions other than traumatic injury to the *nervus ischiadicus* are also contemplated, including mechanical and chemical damage to a plurality of

nerve cells, infection of neuronal cells with bacteria and/or viruses, and degenerative diseases. Regardless of the nature of the patient's condition, it is contemplated that the method according to the inventive subject matter may stimulate a wide range of neuronal cells, and it is especially contemplated that the stimulated neurons communicate between the person's brain and voluntary muscles, or between the persons brain and skin sensors.

Another class of contemplated methods includes improving tactile or other sensory sensitivity in a patient. Still another class of contemplated methods includes improving gross and fine motor control.

While not wishing to be bound to a particular theory, it is contemplated that the administration of Ribavirin, Levovirin™, or mono-, di- and triphosphorylated forms of Levovirin™ or Ribavirin may effect a change in the Type 1 or Type 2 response in a patient, which concomitantly may lead to a neuroprotective status, or a stimulation of neuronal growth. Therefore, it is contemplated that compounds according to the inventive subject matter may be administered as part of a treatment of a disease in a patient in a dosage range effective to increase a Type 1 response and decrease a Type 2 response in the patient. With respect to *in vivo* administration (route, dosage, schedule, term, etc.) of Ribavirin or alternative contemplated compounds, the same considerations as described below apply.

Antineoplastic treatment

It is further contemplated that compounds according to the inventive subject matter may be employed as antineoplastic agent in treatment of a solid or lymphatic tumor, and contemplated neoplasms include various carcinomas, sarcomas, and lymphomas, and particularly include acute myeloid leukemia and chronic myeloid leukemia in blast crisis. It should further be appreciated that administration of contemplated compounds in antiviral treatments will generally follow a route, dosage, schedule and term as employed with known D-nucleotides, D-nucleosides, and their respective analogs in antineoplastic treatments.

Administration of Contemplated Compounds

With respect to administration of contemplated compounds, it should be appreciated that the compounds may be administered under any appropriate protocol in any appropriate pharmaceutical formulation. It is generally preferred that contemplated compounds are orally administered. In alternative aspects of the inventive subject matter, it should be appreciated that various alternative administrations are also suitable, and it should further be recognized that a particular administration will generally depend on chemical stability, bioavailability, dosage, formulation, and/or desired pharmacokinetic/pharmacodynamic properties of contemplated compounds. Thus, appropriate administrations will include topical delivery (*e.g.*, ointment, spray, cream, etc.), parenteral systemic delivery (*e.g.*, inhalation), and direct or indirect delivery to the blood stream (*e.g.*, i.v. or i.m. injection, etc.).

Consequently, the formulation of contemplated compounds may vary considerably. For example, where the drug or drug composition exhibits sufficient stability to pass through the gastro-intestinal system without undesired chemical or enzymatic modification, oral formulations may include syrup, tablets, gel caps, powder, etc. On the other hand, where absorption or passage of contemplated compounds through the gastro-intestinal tract into the blood stream is problematic, suitable formulations especially include injectable solutions or suspensions (*e.g.*, physiological saline solution buffered to a pH of about 7.2 to 7.5).

With respect to the dosage of contemplated compounds, it should be appreciated that various dosages are suitable, and contemplated dosages typically are in the range of 1mg to several 100mg, and even more. For example, where contemplated compounds are excreted or metabolized at a relatively low rate, or where long-term treatment is desired, dosages will typically be in the range between 5mg-200mg per day. On the other hand, where bioavailability of contemplated drugs is relatively low, or where metabolic conversion (*e.g.*, dephosphorylation) is relatively fast, dosages will typically be in the range between 100mg-2500mg per day.

With respect to the dosage of L-nucleosides, and especially Levovirin™, it should further be appreciated that Levovirin™ appears not to be phosphorylated *in vivo*, at least not in hepatocytes and erythrocytes, and since the antiviral effect of Ribavirin appears to be dependent on phosphorylation, one of ordinary skill in the art would not expect Levovirin™ to have a direct antiviral effect. In fact, experiments (not reported) show no direct antiviral effect. The antiviral effect of Levovirin™ surprisingly appears to be at a dosage of not more than 200 mg per day,

preferably in the 10 to 200 mg range, more preferably in the 50 to 200 mg range, and even more preferably in the 50 to 100 mg range. This is supported by experimental evidence demonstrating that a given dose of Levovirin™ results in a serum level five times that of an equivalent dose of Ribavirin.

Experiments show that Ribavirin is removed from serum by becoming phosphorylated in red blood cells (see, *e.g.*, Homma, M. et al.; High-performance liquid chromatographic determination of ribavirin in whole blood to assess disposition in erythrocytes; Antimicrob. Agents Chemother. (1999), 43(11):2716-9). Once phosphorylated, Ribavirin cannot leave the cells. Consequently, red blood cells act as a Ribavirin sink, and higher doses of Ribavirin are needed to achieve a given serum level. Levovirin™ is not phosphorylated, and therefore, tends not to accumulate in red blood cells. As a result, red blood cells do not act as a Levovirin™ sink, and lower doses of Levovirin™ are sufficient to achieve a desired serum level.

The schedule of administration may vary considerably, and contemplated schedules include a single dose over the entire course of treatment, multiple single daily doses over the entire course of treatment, multiple daily doses, and permanent dosing (e.g., permanent infusion, implanted osmotic pump, etc.) for at least part of the course of treatment. While it is generally preferred that suitable schedules sustain constant delivery of contemplated compounds, burst delivery (*i.e.*, at least one administration at a first dose followed by at least one more administration at a dose lower than the first dose) is also appropriate. With respect to the term (*i.e.*, duration) of treatment, it is contemplated that appropriate durations may vary between a single administration and several days, several weeks, several years, and even longer. For example, where contemplated compounds are employed in a cell culture, a single administration, or relatively short administration may be sufficient. On the other hand, where contemplated compounds are administered to treat an acute hepatic disease, appropriate treatment duration may be in the range between several days and several weeks. Similarly, where chronic hepatic diseases are treated by administration of contemplated compounds, extended administration over one or more years may be suitable.

In still further alternative aspects of the inventive subject matter, contemplated compounds may be combined with additional pharmaceutically active substances to assist in the treatment of various diseases, and particularly viral infections. Additional pharmaceutically active substances may be administered separately or together, and when administered separately,

administration may occur simultaneously or separately in any order. Especially contemplated additional pharmaceutically active substances include antiviral agents and immune modulator substances. For example, antiviral agents include protease inhibitors, nucleotide and/or nucleoside analogs (and especially Ribavirin), and immune modulator substances may include cytokines (*e.g.*, interferon α and γ, IL2, IL4, IL6, IL8, IL10, and IL12).

Further contemplated pharmacologically active agents include anti-fungal agents such as tolnaftate, Fungizone™, Lotrimin™, Mycelex™, Nystatin and Amphoteracin; anti-parasitics such as Mintezol™, Niclocide™, Vermox™, and Flagyl™, bowel agents such as Immodium™, Lomotil™, and Phazyme™; anti-tumor agents such as interferon α and γ, Adriamycin™, Cytoxan™, Imuran™, Methotrexate, Mithracin™, Tiazofurin™, Taxol™; dermatologic agents such as Aclovate™, Cyclocort™, Denorex™, Florone™, Oxsoralen™, coal tar and salicylic acid; migraine preparations such as ergotamine compounds; steroids and immunosuppresants not listed above, including cyclosporins, Diprosone™, hydrocortisone; Floron™, Lidex™, Topicort™, and Valisone™; and metabolic agents such as insulin, and other drugs which may not fit into the above categories.

Preferred combinations of contemplated compounds with an interferon

In a particularly preferred aspect of the inventive subject matter, a synergistic combination of Levovirin™ and at least one interferon, preferably IFN-α-2b, is contemplated. Levovirin™ is typically not, or only to a significantly lesser extent than Ribavirin, phosphorylated in erythrocytes while still exhibiting antiviral and immunomodulatory activity. Consequently, it is contemplated that the pharmacological action of interferon, and especially in the treatment of hepatic diseases, can be potentiated by co-administration of Levovirin™ at significantly lower dosages as compared to Ribavirin. For example, it is contemplated that effective synergistic doses of Levovirin™ in combination with interferon needed to treat HCV infection are projected to be in the 1-600 mg range, more preferably in the 10-400 mg range, still more preferably in the 50-300 mg range, and most preferably in the 100-300 mg range. Equivalent synergistic doses of Ribavirin are considered to be 600-800 mg.

In another aspect of the inventive subject matter, it is contemplated that the synergistic combination of Levovirin™ and interferon will result in reduced toxicity relative to a

combination of Ribavirin and interferon at equivalent effective dosages, predominantly due to the lack of significant phosphorylation in erythrocytes. Viewed from yet another perspective, it is contemplated that the synergistic combination of Levovirin™ and interferon specifically allows targeting of the liver due to the lack of phosphorylation of Levovirin™ in compartments other than the liver, especially erythrocytes.

With respect to co-administration of Levovirin™ and interferon, it is contemplated that all suitable routes and protocols are appropriate, and it is especially preferred that Levovirin™ and interferon are administered in a protocol similar to known administration protocols of Ribavirin and interferon. For example, Levovirin™ may be orally administered while Interferon may be subcutaneously injected. In general, it is contemplated that co-administration of Levovirin™ and interferon may utilize schedules and routes independent from each other so long as both drugs are in the blood stream at measurable concentrations at the same time. It is further contemplated that effective dosages of Levovirin™ can be projected from the effective concentrations of Ribavirin in the liver where Ribavirin was administered.

While Levovirin™ is particularly contemplated, chemical modifications including prodrug forms such as modified Levovirin™ (1-beta-L-ribofuranosyl-1,2,4-triazole-3-carboxamidine), mono- di- and triphosphorylated Levovirin™, and stereochemical variants (*e.g.*, enantiomers, isomers, etc.) are also appropriate. Examples for suitable chemical modifications and prodrug forms are described in U.S. Patent Application Number 09/594410, (*supra*). It is still further contemplated, that suitable drugs may also include drugs other than Levovirin™ and its variants, and particularly contemplated alternative drugs include liver specific prodrugs with an amine or amide group that can be enzymatically deaminated/deamidated in the liver.

With respect to the interferon, it is contemplated that co-administration of Levovirin™ need not be limited to IFN-α-2b, and co-administration may also include natural and synthetic fragments, isoforms, and consensus forms of interferon-alpha. Moreover, interferons other than interferon-alpha are also suitable, including interferon-beta and its natural and synthetic fragments, isoforms, and consensus forms. While interferon is particularly contemplated, cytokines other than interferon and chemokines are also appropriate, including IL-2, IL-12, and TNF. It is especially contemplated, that pegylated forms of contemplated interferons (*i.e.* contemplated interferons associated with polyethylene glycol) are also suitable for use in conjunction with the teachings presented herein.

Combination of contemplated compounds with a second compound that binds a viral protein or a cytokine

Where the combination of contemplated compounds with other pharmacologically active agents is employed in an antiviral therapy, it is particularly contemplated that such combinations may comprise contemplated compounds with a direct and an indirect antiviral effect, and a second compound that increases the total antiviral effect (the total antiviral effect includes the direct antiviral effect and the indirect antiviral effect), wherein the second compound specifically binds a viral protein or a cytokine.

With respect to contemplated compounds in the combination, nucleoside analogs are preferred, and it is even more preferred that the nucleoside analog is Ribavirin (1-(5-Deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide). Ribavirin is known to have a direct antiviral effect by inhibiting RNA and DNA virus replication [Huffman et al, *Antimicrob. Agents Chemother* (1973), 3: 235; Sidwell et al., Science (1972), 177: 705] and an indirect antiviral effect by suppressing Type 2 mediated T cell responses and promoting Type 1 mediated T cell responses as described in U.S. Patent Application Number 09/156,646, which is incorporated herein by reference. However, various compounds other than Ribavirin are also contemplated, and particularly include L-nucleoside analogs, so long as such alternative compounds have a direct and an indirect antiviral effect. For example, where especially high concentrations of an L-nucleoside analog are desirable, Levovirin™ may be employed.

It should further be appreciated that depending on the chemical nature of the first compound, the first compound may have a more pronounced direct antiviral effect or a more pronounced indirect antiviral effect. Contemplated direct antiviral effects include inhibition of viral replication, for example, an inhibition of a reverse transcriptase, whereas contemplated indirect antiviral effects include a shift in a Type 1/Type 2 balance towards a Type 1 or Type 2 response as described in U.S. Patent Application Number 09/156,646. It should also be appreciated that an indirect antiviral effect may comprise a suppression of a Type 1 and Type 2 response, which is described in greater detail in U.S. Provisional Patent Application Number 60/172,097 (*supra*). The shift of a Type 1/Type 2 balance towards a Type 1 or Type 2 response or suppression of the Type 1/Type 2 response may be advantageously controlled by the same

first compound, wherein the dosage of the first compound determines the shift or suppression in a Type 1 or Type 2 response.

With respect to the second compound, it is preferred that the second compound comprises an antibody (*e.g.*, a monoclonal or polyclonal antibody). It should be appreciated, however, that in alternative aspects of the inventive subject matter the antibody need not be restricted to a naturally occurring form of an antibody, but may also include a synthetic form of an antibody (*e.g.*, mini antibodies obtained by phage panning, or other molecular evolution technology), or antibody fragments. Antibody fragments are especially desirable, where such fragments are produced by a recombinant cell, or where the molecular weight of the second compound should be relatively low (*i.e.*, below 75kDa). Contemplated antibody fragments include an Fab, an $F(ab)_2$, and an scFab. Furthermore, it is contemplated that appropriate antibodies may be modified to introduce various additional features, including a reporter group, a second affinity moiety (*e.g.*, a bispecific antibody), or a pharmacologically active molecule. For example, a reporter group may include a radioisotope, or a metal that is detectable with *in vivo* scanning devices (*e.g.*, magnetic resonance imaging). Contemplated pharmacologically active molecules may include reverse transcriptase inhibitors, protease inhibitors, or cytotoxic agents. The production of recombinant and non-recombinant antibodies is well known in the art (*e.g.*, see *Current Protocols in Immunology;* John Wiley & Sons (1999); Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober), and it is contemplated that all known methods for their production are suitable for use in conjunction with the teachings presented herein. Antibodies are typically administered by injection (*e.g.*, i.v. injection), and the actual dose will typically lie between 0.01mg and several 10mg, however, where appropriate, lower dosages are also contemplated.

It should further be appreciated that binding of the second compound to a viral protein or a cytokine is particularly advantageous where binding leads to an inactivation of a viral protein and/or a cytokine, and it is contemplated that inactivation may occur via various mechanisms. For example, inactivation of a virus may be achieved by antibody-mediated precipitation (*i.e.*, formation of a molecular network between antibodies and viruses). Alternatively, binding of the second compound may inactivate a virus by blocking or otherwise obstructing proteins or other viral surface structures that are essential to the infectivity or propagation of the virus. Still further, binding of the second compound may occur with non-structural viral proteins, including viral polymerases and proteases. For example, contemplated binding targets (*i.e.*, haptens)

include proteins such as the gp120/41 of a HIV virus, but also proteins such as the reverse transcriptase of the HIV virus. Further contemplated viral proteins include proteins from a HIV virus, a hepatitis virus, an influenza virus, and an RSV virus. With respect to cytokines it is contemplated that inactivation may be achieved by sequestration of the cytokine from the pool of cytokines. For example, where the hapten for contemplated second compounds is a Type 1 cytokine, particularly contemplated cytokines include interleukin-2, interferon-gamma, and tumor necrosis factor-beta, whereas in cases where the hapten for the second compound is a Type 2 cytokine, particularly contemplated cytokines include interleukin-4, interleukin-5, and interleukin-10.

It is generally contemplated that inactivation of a virus or a cytokine by the second compound may have a plurality of desirable effects, which may or may not exhibit an additive or synergistic effect in combination with the first compound. For example, it is contemplated that in cases where the first compound has a direct antiviral effect (resulting in a significant reduction of viral titer), a second compound may even further reduce the virus titer by precipitating remaining viruses. Alternatively, the second compound may reduce the number of infectious virus particles by binding to viral components that are essential for infectivity. It is further contemplated that where the second compound binds a cytokine, the second compound may shift the Type 1/Type 2 balance towards a Type 1 response by sequestering one or more Type 2 cytokines from the pool of cytokines, and thereby helping to restore cellular immunity while the virus load is already significantly reduced. In another example, it is contemplated that where the first compound has an indirect antiviral effect (also resulting in a significant reduction of virus titer), a second compound may further reduce the virus titer by precipitating remaining viruses. Alternatively, one or more Type 1 and/or Type 2 cytokines may be sequestered by a second compound or mixture of second compounds thereby "fine tuning" (*i.e.*, modulating) a Type 1 and/or Type 2 response induced by the first compound.

It should be appreciated that a combination of a first compound that has a direct and indirect antiviral effect with a second compound that specifically binds a virus and/or a cytokine will reduce a viral titer not only by a mechanistic (*i.e.*, enzyme inhibition), but also by a systemic (*i.e.*, stimulation/modulation of immunity) action. It is especially contemplated that preferred antiviral drug compositions include a first and second compound having a synergistic effect, which advantageously will help reduce the effective dosage of the first and second compound. It

is still further contemplated that appropriate antiviral drug compositions may also be employed in a prophylactic treatment.

Combination of contemplated compounds with Ribavirin

It is particularly contemplated that co-administration of Levovirin™ with Ribavirin will reduce adverse side effects and improve tolerability of Ribavirin and/or Levovirin™. With respect to the ratio of Ribavirin to Levovirin™ in the co-administration, it is preferred that Levovirin™ is present in at least an equimolar amount of Ribavirin. However, it should be appreciated that various alternative ratios are also appropriate, and the particular ratio will predominantly depend on the desired effect and dosage/route of administration. For example, where hemolytic anemia is of particular concern, Levovirin™ may be present in the co-administration in a range of about 51mol% to about 80mol%, or more. On the other hand, where tolerability of Levovirin™ is limiting, Levovirin™ may be present in the co-administration in a range of about 49mol% to about 20mol%, or less.

It should further be appreciated that co-administration of Ribavirin and Levovirin™ need not necessarily employ the same route of administration. The term "co-administration" as used herein refers to any form of administration of Ribavirin and Levovirin™ such that Ribavirin and Levovirin™ are present in a measurable concentration in the system at the same time. Therefore, contemplated co-administrations include protocols in which Ribavirin is administered in one route and Levovirin™ is administered in another route, wherein the co-administration may be performed simultaneously or at two different points in time. For example, Ribavirin may be administered orally while Levovirin™ may be injected intravenously. In another example, Ribavirin may be administered orally BID, and Levovirin™ may be administered orally QID.

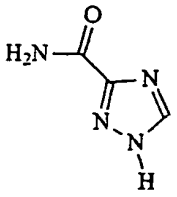
It is particularly contemplated that by variation of the molar fractions of Ribavirin and Levovirin™ in a co-administration protocol, particularly desirable biological effects may be tailored to the specific needs of a patient, including modulation of the Type 1/Type 2 cytokine balance, direct antiviral effect, reduction in hematotoxic properties, etc.

In another aspect of the inventive subject matter, it is contemplated that the administration or co-administration of Ribavirin and Levovirin™ will include a continuous release and/or a reduced dosage at intervals that are more frequent. It is particularly

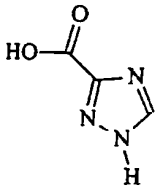
contemplated that continuous release and/or reduced dosage at frequent intervals will reduce undesirable side effects and may increase the direct and/or indirect antiviral effect. While it is generally contemplated that compounds according to the inventive subject matter may be administered to any system, it is preferred that contemplated compounds are administered to a mammal, preferably a human, or to a cell or tissue culture.

Metabolites of Contemplated Compounds

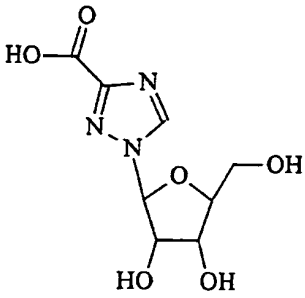
It is generally contemplated that Levovirin™ is metabolically inert when administered to a system, however, the inventors also contemplate that Levovirin™ may have metabolites, which are shown in **Structures 4-8**.



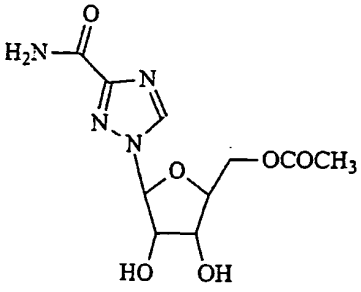
Structure 4



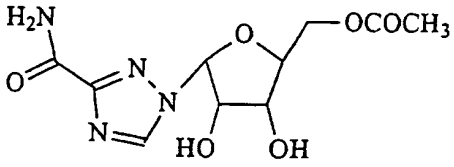
Structure 5



Structure 6



Structure 7



Structure 8

Structure 4 is a triazole carboxamide, **Structure 5** is a triazole carboxylic acid, **Structure 6** is a L-ribofuranosyl triazole carboxamide, **Structure 7** is a 5'-acetyl L-ribofuranosyl triazole carboxamide, and Structure 8 is an 5'-acetyl-α-L-ribofuranosyl triazole carboxamide.

While is it generally contemplated that the metabolic products of Levovirin™ are formed as a product of an enzymatic reaction, it should also be recognized that under suitable intra or extra-cellular conditions in a cellular system, metabolites may be formed without an enzymatic reaction. Thus, formation of metabolites from Levovirin™ may include redox reactions (particularly oxidation), enzymatically catalyzed reactions (*e.g.*, hydrolysis), and photochemical reactions.

Contemplated reaction products are typically degradation products of Levovirin™, however, it should be recognized that metabolites may also include products formed by addition of chemical groups (*e.g.*, glycosylation or acetylation), and that such modified compounds may be subject to subsequent degradation in the same or different compartment. While it is generally contemplated that the metabolites have a significantly reduced pharmacologically effect as compared to Levovirin™, it should be appreciated that the metabolites may have a pharmacological effect similar to Levovirin™. For example, the triazole or ribose moiety may serve as an effector (*e.g.*, allosteric inhibitor).

It is further contemplated that of a particular dose of Levovirin™ administered to a system, between 20% and 50%, preferably between 51% and 75%, more preferably between 76% and 99%, and most preferably 100% are excreted in an unmetabolized form.

Examples

Targeted hepatic immunosuppression employing Ribavirin

Three placebo-controlled studies of Ribavirin in the treatment of chronic hepatitis C were conducted. These studies included 134 patients treated with Ribavirin and 97 patients who received a placebo. There were also two uncontrolled Phase II studies including a total of 23 patients treated with Ribavirin. The primary response parameter was normalization or reduction of serum ALT levels. Response was also assessed in terms of elimination or reduction of serum HCV RNA levels, and improvement in liver histology as assessed by changes in Knodell scores.

In all of the controlled and uncontrolled studies, using the definitions of response specified in the protocols and analysis plans, Ribavirin was statistically significantly superior to a placebo in normalizing and reducing ALT levels during treatment. In the integrated analyses

based on all patients in the controlled studies, using a uniform definition of response including normalization of ALT at the end of treatment or a clinically meaningful level of partial response, 46% of Ribavirin patients were responders compared to 4% of placebo patients ($p<0.001$). Patients generally responded after two to three months of treatment and the response was maintained as long as treatment was continued. There was no evidence of loss of ALT response with increasing duration of treatment. Following withdrawal of Ribavirin at the end of the active treatment phase, 11.5% of responders had a sustained response throughout the follow-up period.

Regarding improvement in liver histology, in each of the controlled studies there was a non-significant trend in favor of Ribavirin in the changes in total Knodell scores and many of the component scores. Analysis of the combined data by analysis of covariance using the baseline Knodell score as covariate resulted in statistically significant differences in favor of Ribavirin for the total score and each of the component scores. Thus in the controlled studies, in comparing all Ribavirin-treated patients with placebo recipients, Ribavirin had a modest but real effect in improving liver histology. Within the Ribavirin group, comparison of ALT responders and non-responders revealed that ALT responders experienced a significantly greater improvement in liver histology as compared to ALT non-responders. The mean fall in total Knodell score was approximately two points for ALT responders as compared to one point for all Ribavirin-treated patients. A fall in a total Knodell score of two points is generally considered by hepatologists to be clinically significant. There was thus a statistically significant positive correlation between ALT response and a clinically significant degree of improvement in liver histology.

In all studies, the primary endpoint was defined as a reduction in ALT level. In all studies a complete ALT response was defined as normalization of the ALT level at the end of treatment. A partial ALT response was defined as either a 50% or greater reduction at the end of treatment from the patient's baseline value, or a 50% or greater reduction to a level not higher than 1.5 times the upper limit of normal.

In studies 92-001 and 91-DK-178, the treatment groups were compared with respect to the effect of the study medication on symptoms relevant to hepatitis. This could not be done in study CT00/002 because the case report form did not permit the systematic collection of symptom data.

In study 92-001, there was a statistically significant difference in favor of Ribavirin for decreased fatigue. At the end of treatment and at the end of the follow-up period, a higher

proportion of Ribavirin patients showed some improvement from baseline in fatigue compared to placebo patients (p=0.04 for end of treatment and p=0.006 for end of follow-up). In this study, there were no significant differences between the treatment groups for any other symptoms.

In study 91-DK-I78, there were isolated significant differences between the treatment groups in individual symptoms at individual visits, some favoring the placebo group and some the Ribavirin group, but there were no overall trends favoring either treatment group.

Table 1 shows the response rates in terms of improvement in liver histology. There were no statistically significant differences between the treatment groups in the changes in Knodell scores in any of the studies, although there were numerical trends in favor of Ribavirin. In study CT00/002 there was a difference in favor of Ribavirin in one of the secondary parameters (lymphoid aggregates, p=0.05).

Protocol	Protocol Definition of Response	Analysis Plan Definition of Response	Result
92-001	Comparison of treatment groups with respect to the changes from pre- to post-treatment in each patient's Knodell scores	Same	No significant difference between treatment groups
91-DK-178	Long-term response: Improvement in liver histopathology by "blinded ranking of all liver biopsies for the degree of current hepatic injury using the Wilcoxon rank sum test"	Comparison of treatment groups with respect to the changes from pre- to post-treatment in each patient's Knodell scores	No significant difference between treatment groups
CT00/002	Improvement in degree of inflammatory activity from pre- to post treatment as assessed by Knodell scores	Comparison of treatment groups with respect to the changes from pre- to post-treatment in each patient' s Knodell scores --- Pre- to post treatment changes in other histological parameters thought to be relevant to hepatitis C	No significant difference between treatment groups --- Reduction in lymphoid aggregates in Ribavirin group

Table 1 - Comparison of Results of Controlled Studies - Liver Histology

Further analyses were performed in respect of the different studies. However, to make the results of these analyses more meaningful, the control studies data were combined to make the sample sizes larger.

Results of Integrated Analyses

Analysis of Response to Ribavirin According to the Relevant Effectiveness Criteria –ALT Response During Ribavirin Therapy

For the purposes of the integrated effectiveness analyses, the following definitions of ALT response were used:

Complete Response: Return to within the normal range at the end of treatment.

Partial Response: 50% or greater reduction from the patient's baseline level to within 1.5 times the upper limit of normal at the end of treatment.

Responder: Meets above definitions of either complete or partial response. The definition of "responder" was determined by plotting the ALT values over time for the groups of patients fitting various definitions of response employed within each study. (The data were fitted with a cubic spline smoothing function Reinsch 1967). Three definitions of response were used:

a. Complete response = ALT in normal range at end of treatment.

b. Partial response (A) = 50% or greater reduction from the patient's baseline level to within 1.5 times the upper limit of normal at the end of treatment.

c. Partial response (B) = 50% or greater reduction from the patient's baseline level at the end of treatment.

All other patients were considered non-responders. Plots were also constructed for Ribavirin-treated non-responders within each study and for all placebo patients (responders and non-responders) within each study.

The three curves for "complete response" demonstrated that this response was achieved after approximately one third of the treatment period and was maintained thereafter. The three curves for "partial response (A)" demonstrated a similar pattern of response. The three curves for "partial response (B)" demonstrated distinctly more variability of ALT levels during the treatment periods. The plots for Ribavirin-treated non-responders and the plots for placebo patients demonstrated, as expected, a dispersion of the data points which did not change in any recognizable pattern across the treatment and follow-up periods. It was decided that the "partial

response (B)" definition was inappropriate for the purpose of the integrated effectiveness analyses. Due to the consistent pattern of response demonstrated by the "complete response" and "partial response (A)" definitions, and the fact that these definitions are clinically meaningful, it was decided that for the purpose of the integrated effectiveness analyses a "response' be defined as either "complete response" or "partial response (A)".

Table 2 displays the results for each study and for the combined database, using the above definition of ALT response. The proportions of responders in the two treatment groups were compared using either a Chi-square or Fisher's Exact test.

Study	Ribavirin n/N (%)	Placebo n/N (%)	p Value
92-001	15 / 28 (53.6)	1/30 (3.3)	<0.001
91-DK-178	11/29 (37.9)	1/29 (3.4)	<0.001
CT00/002	32/70 (45.7)	2/36 (5.6)	<0.001
Combined database	58/127 (45.7)	4/95 (4.2)	<0.001

n = Number of patients with an ALT response (integrated definition) N = All patients treated (intent-to-treat population) minus those without valid non-missing observations.

Table 2: Percent ALT Response Rates

For identifying the ALT value corresponding to the end of treatment, the last valid, non-missing observation, going back a maximum of two visits, was carried forward for those patients missing such a true value. This same policy was employed in two out of the three controlled studies (91-DK-178 and CT00/002). Nine patients in the Ribavirin group and two placebo patients did not have such a valid non-missing observation available. The ALT response rates were consistent across the three controlled studies and ranged from 37.9 to 53.6%. When the data were combined, the ALT response rate was 45.7%. In all instances, the ALT response rates for patients treated with Ribavirin were statistically, significantly superior to the rates in patients treated with placebo. In the placebo group, ALT response rates were consistently low, and ranged from 3.3 to 5.6% and 4.2% when the data were combined.

ALT Response in the Follow-Up Period

Table 3 summarizes the rates of sustained response in studies 92-001 and 91-DK-178 and in these two studies combined. The individual study, analysis plan definitions of sustained response are used. A sustained responder is essentially a patient with either normalization of ALT or a partial response at the end of treatment, who still meets either of these criteria throughout the follow-up period. It was not possible to provide this same analysis for study CT00/002 because too few patients had complete ALT data throughout the follow-up period.

Study	Ribavirin n/N (%)	Placebo n/N (%)
92-001	1/15 (6.7)	0/1 (0.0)
91-DK-178	2/11 (18.2)	0/1 (0.0)
Combined database	3/26 (11.5)	0/2 (0.0)

n= Number of patients with sustained ALT response N = Number of patients with complete or partial response at the end of treatment (study analysis plan definitions)

Table 3: Percent ALT Sustained Response Rates - Protocol Definitions

In the two studies analyzed, the sustained ALT response rates were 6.7 and 18.2% for Ribavirin-treated patients compared with 0% for patients receiving placebo. Due to the low sample size and the very low number of placebo responders a statistical analysis was not performed.

Improvement in Knodell Scores

Within each of the three controlled studies, there was a consistent numerical trend in favor of Ribavirin in the changes in total scores and in many of the component scores. This same trend was thus apparent when the data from the three studies was combined. The trend applies not only to improvement of scores but also to worsening, indicating that even if no patients in either treatment group improve, then fewer patients in the Ribavirin group are worsening. This is an important observation considering that one objective of treatment is to prevent deterioration of a chronic and progressive condition. Analysis of the combined data by the CMH chi-square test does not reveal any statistically significant differences. Analysis of the combined data by analysis of variance (as used in studies 92-001 and 91-DK-178) revealed a statistically significant difference in favor of Ribavirin for the total Knodell score but not for any of the component scores.

The liver histology data was examined further by analysis of covariance, using the baseline Knodell score as covariate. Regression analysis of the baseline Knodell scores versus the end of treatment scores for all Ribavirin-treated and placebo patients combined resulted in a slope of less than 1.0 but greater than zero. This indicated that the baseline Knodell score influenced the expectation of outcome of treatment, regardless of any difference between Ribavirin and placebo. Where the regression slope differs markedly from 1.0, analysis of covariance is a more appropriate test than analysis of variance (Fisher 1951). The result of the analysis of covariance is displayed in Table 4. The mean changes in the scores for Ribavirin-treated patients are only small, but due to small variances, the differences from placebo are statistically significant. It is of interest to note that the only Knodell sub-score that does not

improve is fibrosis, and that there is less deterioration in the Ribavirin group than in the placebo group.

	Mean Change in Score from Baseline to End of Treatment		
Knodell Component	Ribavirin N = 107	Placebo N = 78	p Value
Periportal Activity and Necrosis	-0.40	-0.01	0.0004
Portal Inflammation	-0.30	-0.10	0.0206
Lobular Necrosis	-0.33	-0.13	0.0019
Fibrosis	+0.07	+0.25	0.0071
Total score	-1.11	-0.05	0.0091

Table 4: Comparison of Ribavirin and Placebo in Terms of Changes in Knodell Scores from Baseline to End of Treatment – Analysis of Covariance Using Baseline Knodell Score as Covariate All Phase III Studies Combined

Examination of Correlation between ALT Response and Improvement in Knodell Scores

In each of the three controlled studies, Ribavirin was significantly more effective than placebo in normalizing and reducing ALT levels (An elevated serum ALT level is a biochemical indicator of hepatic inflammation). There was also a correlation between response to Ribavirin therapy in terms of normalization or reduction of ALT level within individual patients, and by an improvement in liver histology as determined by Knodell scores. We found that there was indeed a consistent trend towards a positive relationship between ALT response and improvement in Knodell scores when both parameters are treated in a categorical manner. In the combined database, this trend was statistically significant for the Total Knodell score ($p = 0.008$), Fibrosis ($p = 0.014$), and Portal Inflammation ($p = 0.022$) using the Cochran-Mantel-Haenszel (CMH) chi-square test (**Table 5-7**).

		Responder		Non-Responder			
Protocol	Response	n	%	n	%	P-value[1]	
92-001	Improved	7	46.7	4	33.3	0.099	
	Unchanged	7	46.7	3	25.0		
	Worsened	1	6.7	5	41.7		
91-DK-178	Improved	10	90.9	7	38.9	0.021	*
	Unchanged	1	9.1	5	27.8		
	Worsened	0	0.00	6	33.3		
CT00/002	Improved	11	42.3	12	48.0	0.171	
	Unchanged	11	42.3	5	20.0		
	Worsened	4	15.4	8	32.0		
Integrated	Improved	28	53.8	23	41.8	0.008	*
	Unchanged	19	36.5	13	23.6		
	Worsened	5	9.6	19	34.5		

N= Number of patients in study n= Number of patients in response category %=(n/N)*100 [1] CMH statistics * P<0.05

Table 5: Knodell Response Status by Controlled Studies – Total by ALT Response

		Responder		Non-Responder			
Protocol	Response	n	%	n	%	P-value[1]	
92-001	Improved	2	13.3	1	0.00	0.434	
	Unchanged	12	80.0	11	91.7		
	Worsened	1	6.7	1	8.3		
91-DK-178	Improved	5	45.5	2	11.1	0.119	
	Unchanged	4	36.4	11	61.1		
	Worsened	2	18.2	5	27.8		
CT00/002	Improved	6	23.1	1	4.0	0.120	
	Unchanged	18	69.2	20	80.0		
	Worsened	2	7.7	4	16.0		
Integrated	Improved	13	25.0	3	5.5	0.014	*
	Unchanged	34	65.4	42	76.4		
	Worsened	5	9.6	10	18.2		

N= Number of patients in study n= Number of patients in response category %=(n/N)*100 [1] CMH statistics
* P<0.05

Table 6: Knodell Response by Controlled Studies – Fibrosis by ALT response

		Responder		Non-Responder			
Protocol	Response	n	%	n	%	P-value[1]	
92-001	Improved	5	33.3	3	25.0	0.220	
	Unchanged	9	60.0	5	41.7		
	Worsened	1	6.7	4	33.0		
91-DK-178	Improved	5	45.5	4	22.2	0.227	
	Unchanged	6	54.5	11	61.1		
	Worsened	0	0.00	3	16.7		
CT00/002	Improved	5	19.2	6	24.0	0.159	
	Unchanged	21	80.8	16	64.0		
	Worsened	0	0.00	3	12.0		
Integrated	Improved	15	28.8	13	23.6	0.022	*
	Unchanged	36	69.2	32	58.2		
	Worsened	1	1.9	10	18.2		

N= Number of patients in study n= Number of patients in response category %=(n/N)*100 [1] CMH statistics
* P<0.05

Table 7: Knodell Response Status by Controlled Studies –Portal Inflammation by ALT Response

The relationship between ALT response and improvement in liver histology was studied further in order to quantify the improvement in liver histology in patients responding to Ribavirin, and to determine if there was a subgroup of patients who derive a more substantial clinical benefit from treatment with Ribavirin. In each of the three controlled studies, Ribavirin-treated ALT responders and non-responders were compared in terms of the mean changes in total Knodell scores over the course of treatment. This analysis, displayed in **Table 8** thus quantifies the directional changes displayed in Table 5.

It can be seen that in each study there was a correlation between ALT response and improvement in liver histology, in that ALT response was associated with a greater improvement in liver histology as compared to ALT non-responders. This effect was particularly striking in study 91-DK-178, where there was a mean Knodell score reduction of 4.09 in ALT responders

as compared to 0.17 in ALT non-responders. A regression model was employed to examine the relationship between ALT response and Knodell score changes. This revealed that in all three studies ALT Response was a significant predictor of improvement in liver histology. Thus, ALT normalization or reduction correlates with improvement in liver histology in patients with hepatitis C treated with Ribavirin, and patients who achieve an ALT response are likely to derive a substantial clinical benefit. In patients treated with Ribavirin who do not achieve an ALT response, there does not appear to be any clinical benefit in comparison to patients who received placebo.

Study	CT00/002	92-001	91-DK-178	Combined Database
Duration of treatment (week)	24	36	48	
Mean Knodell Change (Range) in ALT Responders*	-1.23 (-7.4) n = 26	-1.47 (-8.2) n = 15	-4.09 (-9.0) n = 11	-1.90 (-9.4) n = 52
Mean Knodell Change (Range) in Non-Responders	-0.96 (-9.4) n = 25	+0.58 (-3.6) n = 12	-0.17 (-6.7) n = 18	-0.36 (-9.7) n = 55
P-Value**	0.004	0.0001	0.002	0.0001
Mean Knodell Change (Range) all Ribavirin Patients	-1.10 (-9.4) n = 51	-0.56 (-8.6) n = 27	-1.65 (-9.7) n = 29	-1.11 (-9.7) n = 107
Mean Knodell Change (Range) all Placebo Patients	-0.09 (-4.4) n = 23	-+0.44 (-3.5) n = 27	-0.52 (-8.4) n = 27	-0.51 (-8.5) n = 77
P-Value	NS	NS	NS	0.01

* ALT response was defined as normalization at the end of treatment or reduction of 50% or more from baseline to within 1.5 times the upper limit of normal at the end of treatment.
** Regression analysis

Table 8

Thus, in summary, the phase III program on Ribavirin in chronic hepatitis C consisted of three randomized, double blind, placebo-controlled, parallel group studies. 134 total patients were randomized to receive Ribavirin and 97 to receive placebo. Response to treatment was assessed using three parameters:

1. Reduction or normalization of the ALT level (elevation of ALT is a biochemical marker of hepatic inflammation).

2. Improvement in liver histology as evidenced by a reduction in the Knodell score (the Knodell scoring system quantifies the degree of liver damage by assigning scores to various relevant microscopic characteristics and summing these sub-scores to give a total score).

3. Elimination of hepatitis C virus from the blood, or a reduction in the amount of virus present.

Of the 134 patients in the phase III studies, there were 101 with complete data on ALT levels, Knodell scores and virus levels. Among these 101 patients, 24 patients met the criteria for an optimal clinical response to Ribavirin therapy. The criteria are normalization or clinically significant reduction of the ALT level, and reduction in total Knodell score of two or more points. For these 24 patients, the clinical response was obtained without an accompanying reduction in the virus level in the blood. **Table 9** below summarizes the data on ALT levels and Knodell scores for the 24 responding patients as compared to the remaining 77 patients who demonstrated lesser degrees of response.

	Responders N=24	Non-Responders N=77
Mean baseline ALT (range) in U/L	142.5 (52-269)	176.9 (35-629)
Mean end-of-treatment ALT (range)* U/L	36.8 (21-62)	91.7 (13-286)
Mean Knodell score reduction (range)	4.1 (2-9)	0.15 (-7 to+9)
* upper limit or normal is 40 U/L		

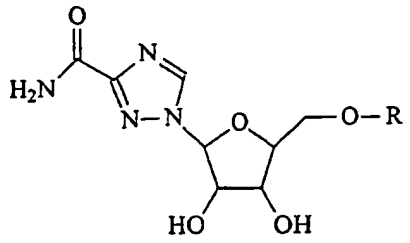
Table 9

Thus, specific embodiments and applications of nucleosides, nucleotides, and their analogs have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

CLAIMS

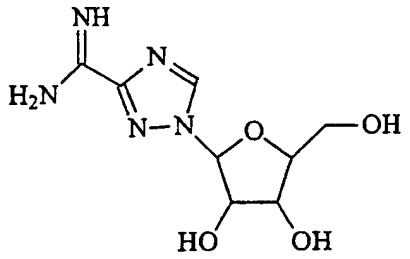
What is claimed is:

1. A compound having the structure:

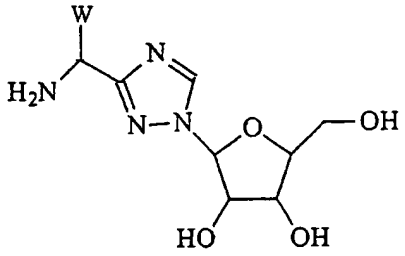


wherein R is PO_3^{2-}, $(PO_3)_2^{3-}$, or $(PO_3)_3^{4-}$.

2. A compound having the structure:



3. A compound having the structure:



wherein W is $-N(R_1)(R_2)$ or $=NR_1$, wherein R_1 and R_2 are independently hydrogen, a linear alkyl, a branched alkyl, an alkenyl, an alkynyl, an aralkyl, an aralkenyl, an aralkynyl, or an aryl.

4. The compound of claim 3 wherein R_1 or R_2 independently further comprises a nitrogen atom, an oxygen atom, a sulfur atom, or a halogen atom.

5. A method of treating a viral infection in a patient comprising administering a composition that includes a compound according to claim 1, claim 2, claim 3, or claim 4 at a dosage effective to inhibit viral propagation.

6. The method of claim 5 wherein the dosage is between 50-500mg/day.

7. The method of claim 5 wherein the dosage is between 500-2500mg/day.

8. The method of claim 5 wherein the viral infection is selected from the group consisting of an HIV infection, an HCV infection, an HBV infection, an RSV infection, an influenza virus infection, and a parainfluenza virus infection.

9. The method of claim 5 further comprising co-administering to the patient a cytokine.

10. The method of claim 9 wherein the cytokine is an interferon.

11. The method of claim 10 herein the interferon is interferon alpha-2b.

12. The method of claim 5 further comprising administering Ribavirin.

13. The compound of claim 1 wherein R is PO_3^{2-}.

14. A method of treating a viral infection in a patient comprising co-administering a composition comprising a compound according to claim 13 at a dosage effective to inhibit viral propagation.

15. The compound of claim 1 wherein R is $(PO_3)_2^{3-}$.

16. A method of treating a viral infection in a patient comprising administering a composition comprising a compound according to claim 15 at a dosage effective to inhibit viral propagation.

17. The compound of claim 1 wherein R is $(PO_3)_3^{4-}$.

18. A method of treating a viral infection in a patient comprising administering a composition comprising a compound according to claim 17 at a dosage effective to inhibit viral propagation.

19. A method of increasing selectivity of a pharmacologically active molecule with respect to a pharmacological effect in a target cell, comprising:

providing a drug, wherein the drug is 1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide;

modifying the drug with a modifying group, wherein the modifying group is covalently attached to the drug via a nitrogen atom; and

wherein the modifying group is enzymatically removed from the drug in the target cell.

20. The method of claim 19 wherein the modifying group is =NH, and wherein the modifying group is covalently bound to a carbonyl atom in the drug.

21. The method of claim 19 wherein the modifying group is $-N(R_1)(R_2)$ or $=NR_1$, wherein R_1 and R_2 are independently hydrogen, a linear alkyl, a branched alkyl, an alkenyl, an alkynyl, an aralkyl, an aralkenyl, an aralkynyl, or an aryl.

22. The method of claim 21 wherein R_1 or R_2 independently further comprises a nitrogen atom, an oxygen atom, a sulfur atom, or a halogen atom.

23. A method of treating a disease characterized by inflammation of an organ in a patient, comprising:

providing a compound according to claim 1, claim 2, or claim 3; and

administering the compound to the patient at a dosage that (a) causes systemic immunomodulation and not systemic immunosuppression of Type I and Type II responses, and (b) causes immunosuppression of Type I and Type II responses in the organ of the patient.

24. The method of claim 23 wherein the compound is a compound according to claim 1.

25. The method of claim 23 wherein the compound is a compound according to claim 2.

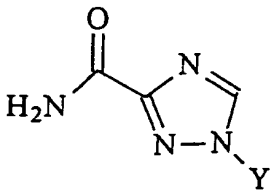
26. The method of claim 23 wherein the compound is a compound according to claim 3.

27. The method of claim 23 wherein the organ is a liver, and wherein the liver is infected with a virus.

28. The method of claim 27 wherein the virus is an HCV virus.

29. A method of stimulating neuronal growth, comprising:

recognizing that a compound having structure I is effective to stimulate growth of neurons within a given concentration range;



Structure I

wherein Y is a 1-β-L-ribofuranosyl, a 1-β-L-ribofuranosyl-5-phosphate, a 1-β-L-ribofuranosyl-5-diphosphate, a 1-β-L-ribofuranosyl-5-triphosphate, a 1-β-D-ribofuranosyl, a 1-β-D-ribofuranosyl-5-phosphate, a 1-β-D-ribofuranosyl-5-diphosphate, or a 1-β-D-ribofuranosyl-5-triphosphate; and

providing the neurons with the compound within the given concentration range.

30. The method of claim 29 wherein the compound is Ribavirin.

31. The method of claim 29 wherein the compound is a phosphorylated Ribavirin.

32. The method of claim 29 wherein the compound is Levovirin™.

33. The method of claim 29 wherein the compound is a phosphorylated Levovirin™.

34. The method of claim 29 wherein the compound is administered as part of a treatment of a disease in a patient, in a dosage range effective to increase a Type 1 response and decrease a Type 2 response in the patient.

35. The method of claim 29 further comprising targeting unipolar neuronal cells in the neurons.

36. The method of claim 29 further comprising targeting bipolar neuronal cells in the neurons.

37. The method of claim 29 wherein the neurons are part of a neuronal tissue including at least four of the following cell types: an astrocyte, a dendrocyte, a myelin sheath cell, a glia cell, a unipolar neuronal cell, a bipolar neuronal cell, a multipolar neuronal cell, and a receptor cell.

38. An antiviral drug composition, comprising:

a first compound having a direct antiviral effect and an indirect antiviral effect; and

a second compound that increases a total antiviral effect, wherein the total antiviral effect includes the direct antiviral effect and the indirect antiviral effect, and wherein the second compound specifically binds a hapten selected from the group consisting of a viral protein and a cytokine.

39. The antiviral drug composition of claim 38 wherein the first compound comprises a nucleoside analog.

40. The antiviral drug composition of claim 38 wherein the nucleoside analog is Ribavirin.

41. The antiviral drug composition of claim 38 wherein the nucleoside analog is Levovirin™.

42. The antiviral drug composition of claim 38 or claim 39 wherein the nucleoside analog is in the form of a prodrug.

43. The antiviral drug composition of claim 38 wherein the direct antiviral effect comprises an inhibition of a viral replication.

44. The antiviral drug composition of claim 38 wherein the indirect antiviral effect comprises a shift of a Type 1/Type 2 balance towards a Type 1 response.

45. The antiviral drug composition of claim 38 wherein the indirect antiviral effect comprises a suppression of a Type 1 and Type 2 response.

46. The antiviral drug composition of claim 38 wherein the second compound comprises an antibody.

47. The antiviral drug composition of claim 46 wherein the antibody is selected from the group consisting of a monoclonal antibody, a polyclonal antibody, a synthetic antibody, and an antibody fragment.

48. The antiviral drug composition of claim 38 wherein the viral protein is a protein of a virus selected from the group consisting of an HIV virus, a hepatitis virus, an influenza virus, a parainfluenza virus, and a RSV virus.

49. The antiviral drug composition of claim 38 wherein the viral protein is a reverse transcriptase.

50. The antiviral drug composition of claim 38 wherein the cytokine is a Type 1 cytokine.

51. The antiviral drug composition of claim 38 wherein the first compound and the second compound have a synergistic effect.

52. The antiviral drug composition of claim 38 wherein at least one of the first compound and the second compound selectively accumulates in an organ.

53. The antiviral drug composition of claim 52 wherein the organ is selected from the group consisting of a liver and a brain.

54. The antiviral drug composition of claim 38 wherein the first compound is Ribavirin and the second compound is an antibody that specifically binds a Type 2 cytokine.

Figure 1A

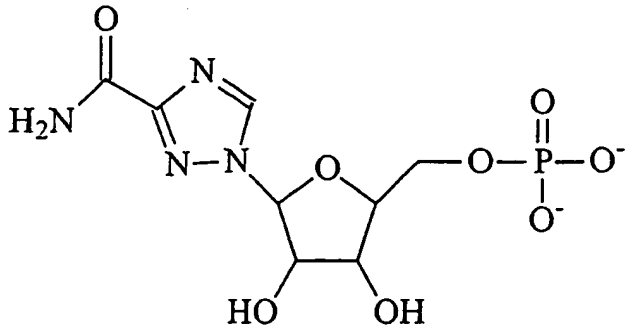


Figure 1B

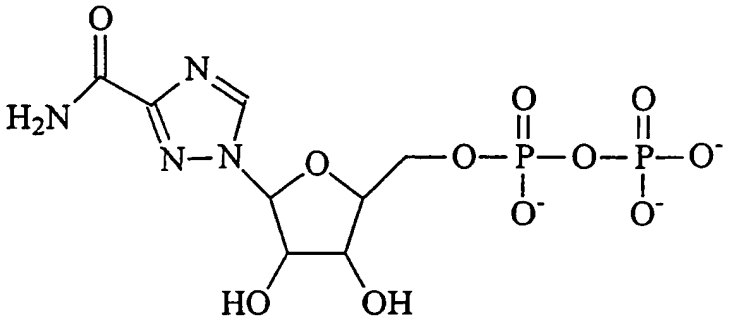
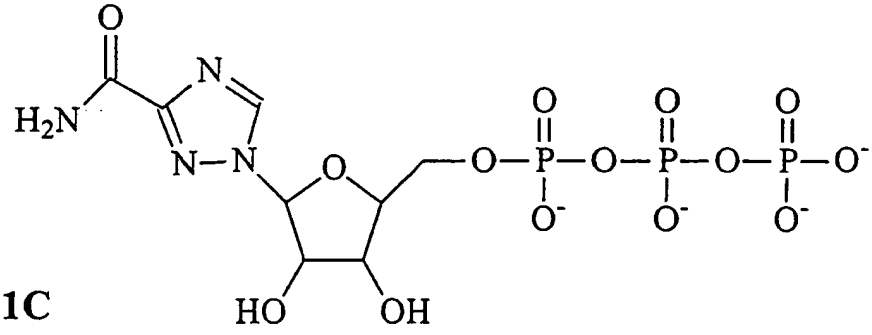


Figure 1C



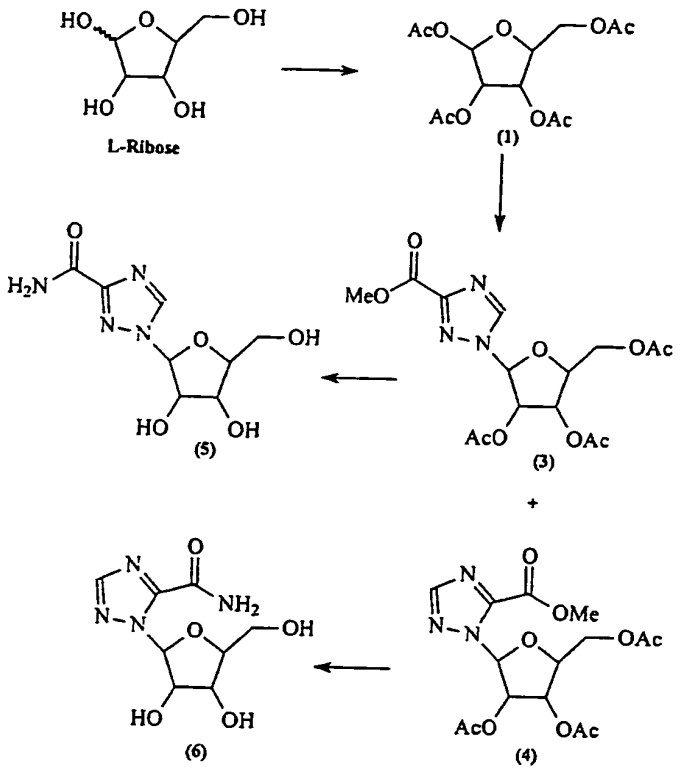


Figure 2

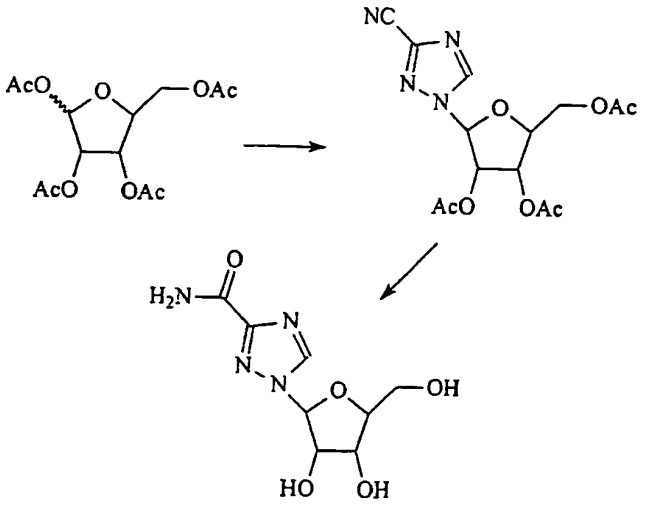


Figure 3

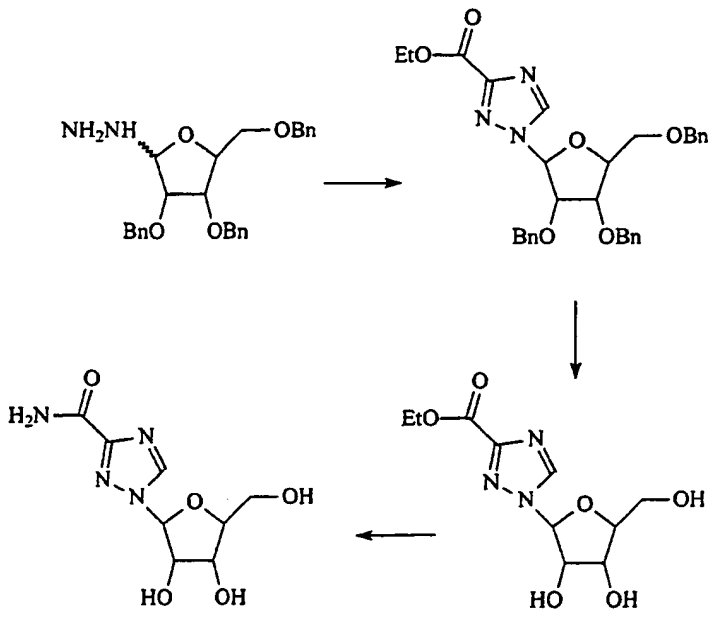

NH2NH
O
OBn
BnO
OBn
EtO
O
N
N
N
O
OBn
BnO
OBn
EtO
O
N
N
N
O
OH
HO
OH
H2N
O
N
N
N
O
OH
HO
OH

Figure 4

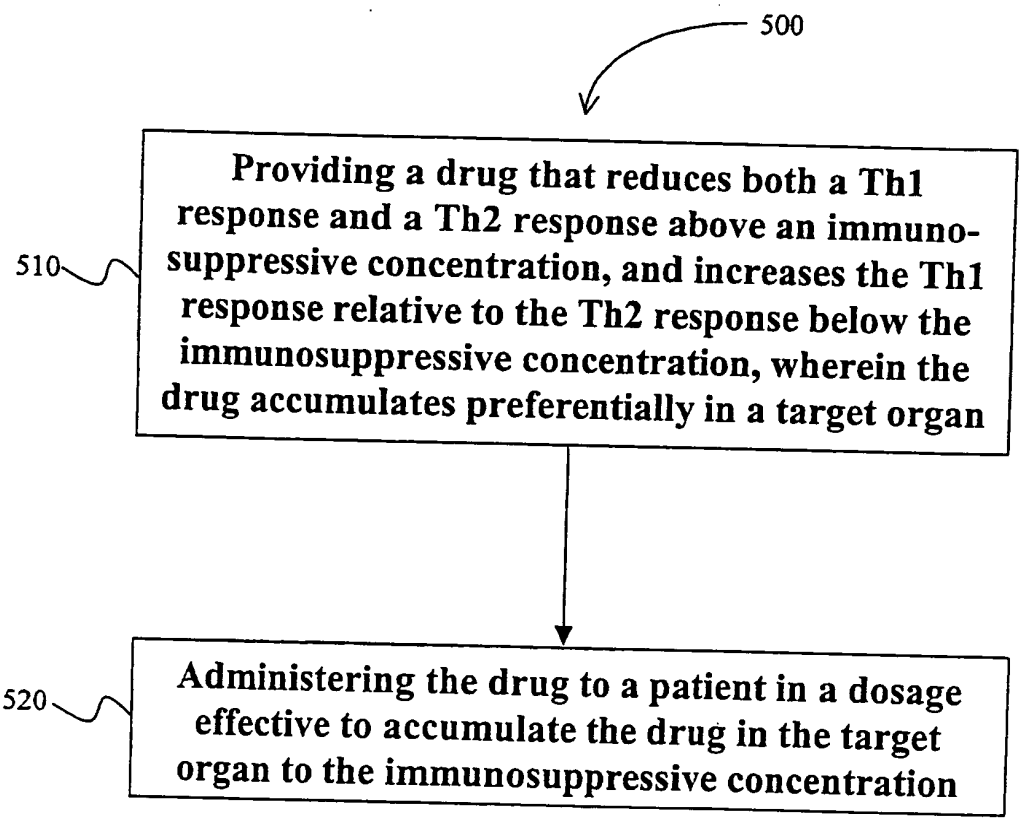

500
Providing a drug that reduces both a Th1 response and a Th2 response above an immuno-suppressive concentration, and increases the Th1 response relative to the Th2 response below the immunosuppressive concentration, wherein the drug accumulates preferentially in a target organ
510
Administering the drug to a patient in a dosage effective to accumulate the drug in the target organ to the immunosuppressive concentration
520

Figure 5

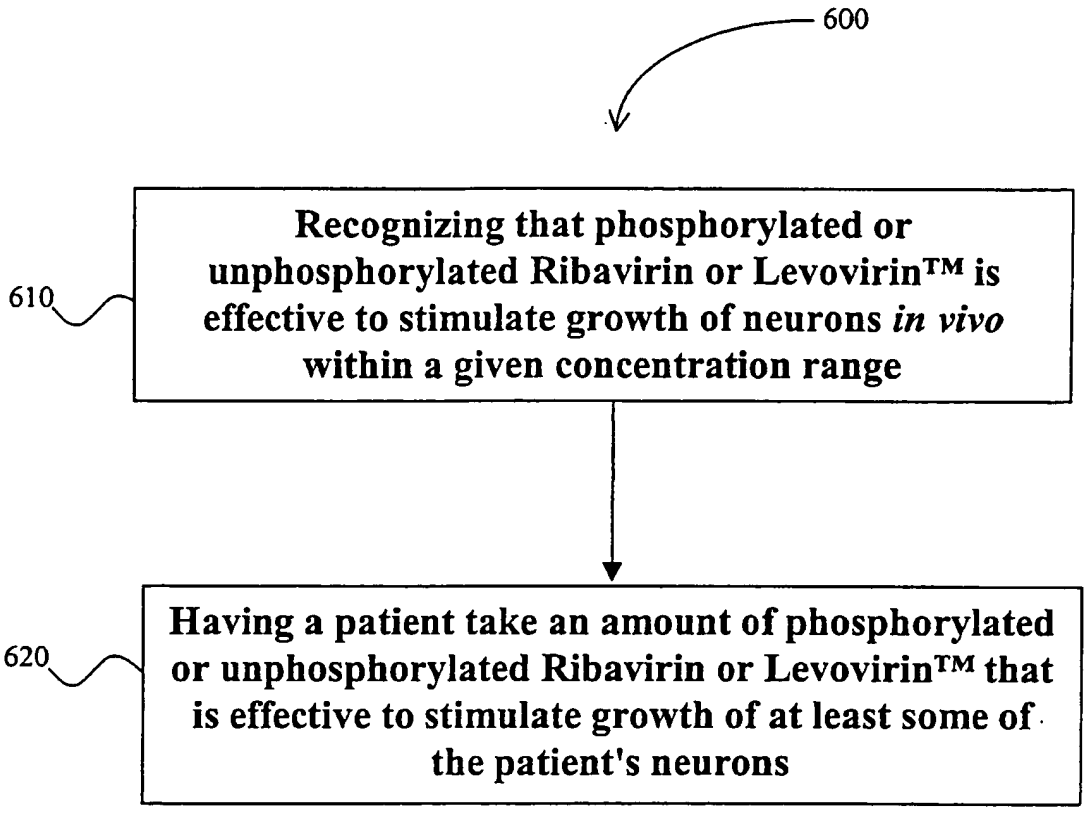

600
610
Recognizing that phosphorylated or unphosphorylated Ribavirin or Levovirin™ is effective to stimulate growth of neurons *in vivo* within a given concentration range
620
Having a patient take an amount of phosphorylated or unphosphorylated Ribavirin or Levovirin™ that is effective to stimulate growth of at least some of the patient's neurons

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/34605

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01N 43/04; A61K 31/70; C07H 19/00; C07H 19/04; C07H 19/044.
US CL : 514/43,49; 536/22.1,26.1,26.9.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/43, 49; 536/22.1, 26.1, 26.9.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database CAPLUS on STN (Columbus, OH, USA), No. 117:131480, Synthesis and antiviral evaluation of N-carboxamidine-substituted analogs of 1-.beta.-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride', abstract, Gabrielsen et al., 1992.	1-23, 27-54
X	WILLIS et al. An in Vivo and in Vitro Evaluation of 1-B-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine: An Inhibitor of Human Lymphoblast Purine Nucleoside Phosphorylase. Molecular Pharmacology. 1980, 18, pages 287-295, especially page 287.	2
X	Database CAPLUS on STN (Columbus, OH, USA), No. 99:47578, 'Inhibition of mRNA methylation: an approach to specific inhibition of viral replication', abstract, Sharma et al., 1982.	1-23, 27-54
X	ALLEN et al. Synthesis and Antiviral of Some Phosphates of the Broad-Spectrum Antiviral Nucleoside, 1-ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin). Journal of Medicinal Chemistry. 1978, Vol.21, No.8, pages 742-746, especially page 742.	1-23, 27-54
A	WO 00/23455 A1 (SCHERING CORPORATION) 27 April 2000 (27-04-2000), see abstract.	1-23, 27-54
A	WO 97/21452 A2 (ADVANCED MAGNETICS,INC) 19 June 1997 (19-06-1997), see abstract.	1-23, 27-54

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
06 February 2001 (06.02.2001)

Date of mailing of the international search report
31 MAY 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Inte .ional application No.
PCT/US00/34605

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 24-26.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/34605

Continuation of B. FIELDS SEARCHED Item3: CAS on line, EAST. search terms used: ribavirin, ribavirin and viral infections, ribavrin and therapeutically effective, ribavirin triphosphate, ribavirin and HIV virus, hepatitis virus, ribavirin derivatives and cytokine.